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INDUCED CHANGES IN THE CELLS OF THE OVOTESTIS OF THE SLUG, *DEROCERAS RETICULATUM* (MÜLLER), WITH SPECIAL REFERENCE TO THE NUCLEOLUS[1]

BY HOPE B. BRIDGEFORD[2] AND D. PELLUET[3]

Abstract

The germinal epithelium lining the ovotestis of the slug, *Deroceras reticulatum*, gives rise to spermatozoa, eggs, and nurse cells. The behavior of the nucleolus has been followed in these cells, particularly in the development of the oocytes. Changes in size, number, and staining reactions of the nucleolar components occur during the rapid growth of the egg. Exposure of animals to low temperature (0° C.) causes two changes in the germinal epithelial cells. The normal flattened cells with elliptical nuclei now become greatly elongated towards the lumen of the gonad and their nuclei become rounded and conspicuous. The ovotestis shows other changes as a result of exposure to cold, such as loss of organization and, in extreme cases, complete disappearance of the cytoplasm of the eggs, leaving only the nuclei. These changes are accompanied by changes in the behavior of the nucleolus. Similar effects of disorganization and loss of cytoplasm follow the injection of an anterior pituitary hormone "Synapoidin") into slugs, but there was no effect on the germinal epithelium. The number of eggs per follicle increased with the number of injections of the hormone. The general effects of these two agents resemble an induced premature maturation followed by senescence. It is suggested that this may be due to interference with the enzyme or hormonal systems of the normal cells.

Introduction

The hermaphroditic land mollusks, including such genera as *Helix*, *Arion*, and *Deroceras*, offer excellent material for the study of differentiation during the life of the animal, since the germinal epithelium lining the ovotestis gives rise to spermatozoa, oocytes, and nurse cells, all of which may be present in the same follicle at one time.

Thus the germinal epithelium may be considered as being made up of indifferent cells, which can be directed along any one of three paths. Since there is no evidence that there is any chromosomal basis for the differentiation of these germ cells, it has been looked for in the cytosome. A voluminous literature dealing with this subject exists, but no changes in the cytosomal inclusions, such as mitochondria or golgi, can be definitely seen in the germinal epithelium which make it possible to predict the future destiny of the cell. The earlier work is reviewed in the careful papers of Ancel (1) and Gatenby(7) The nucleolus of the growing oocyte of these hermaphroditic animals, a most conspicuous nuclear component which goes through a definite cycle paralleling

[1] *Manuscript received June 26, 1952.*
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the rapid growth of the egg, has also been described but its function was until quite recently not understood. Caspersson (4) has established the fact that the nucleolus contains ribonucleic acids and he suggests that the principal function of the nucleolus is the synthesis of cytoplasmic proteins.

The purpose of the present paper was to determine whether the direction of differentiation of the germinal epithelium could be influenced by external and internal factors and to find out whether the nucleolus reflects these changed conditions of the ovotestis. A brief account of the normal behavior of the nucleolus, as we have observed it in the genus *Deroceras*, is necessary for comparison with the experimental material.

Material

Deroceras reticulatum (Müller) is not a native of North America, according to Pilsbry (14), but has been introduced from Europe. The distribution of slugs in Nova Scotia and the histology of their reproductive organs have been described by Ord and Watts (11). The animals were collected in all months of the year, chiefly in Halifax County. The slugs lay large numbers of small transparent eggs, usually in clusters of 2 to 50 or more, and these have been found at all seasons. The eggs hatch from four days to a month after being laid. At this stage of the young slug, the ovotestis consists of a single follicle, covered by thin connective tissue and lined by germinal epithelial cells, the lumen of the follicle being filled with spermatogonia. As the gonad increases in size, other follicles are developed which contain nurse cells, oocytes, and spermatogonia.

Methods

The animals were decapitated, allowing the viscera to be extruded by the contractions of the body wall, after which the gonad was exposed, quickly removed from its attachment on the surface of the digestive gland, and placed in the fixatives. Control and experimental animals were handled in the same way and the same cytological methods were used. Two fixatives were used:— Baker's formal calcium and Zenker's, followed by the staining methods listed below:

(*a*) Baker's formal – sudan black (Baker (2)). Sudan black stains only lipoids, which appear blue-black in the preparation.

(*b*) Mann's methyl blue eosin (Pantin (12)). The method distinguishes basophil from oxyphil elements, the former appearing blue and the latter red. This stain was found by Ludford (10) to be most useful in the study of nucleoli.

(*c*) Feulgen's leucobasic fuchsin and light green (De Tomasi (6)). Chromatin is stained a bright reddish-purple color and the cytoplasmic constituents stain a pale green. Thymonucleic acid is hydrolyzed by dilute hydrochloric acid, producing an aldehyde group which takes up the stain, causing the purple color of the chromatin.

TABLE I

STAINING REACTIONS OF NUCLEOLI AND CHROMATIN

(Zenker fixation, except (*a*) which is Baker's fixative)

	Chromatin					
Stain	Stage	Form	Color	Larger nucleoli	Smaller nucleoli	Spheres
(*a*) Sudan black	Adult oocyte	—	—	Colorless	Colorless	
(*b*) Mann's methyl blue eosin	Undifferentiated germ cells	Blocks	Purple	Red		
	Oogonia	Strands with beads	Purple	Red	Red	
	Adult oocytes	Minute threads joining granules	Purple	Blue or purple occasionally with red areas	Red	Red
	Spermatocytes	Large granules, tiny threads	Blue	Red	Red	
(*c*) Feulgen and light green	Undifferentiated germ cells	Blocks	Purple	Colorless		
	Oogonia	Strands with beads	Purple	Colorless	Colorless	
	Adult oocyte	Very minute threads	Purple	Colorless	Colorless	Colorless
	Spermatocyte	Large granules, tiny threads	Purple	Colorless	Colorless	
(*d*) Feulgen and light green modification	Adult oocyte	Very minute threads	Purple	Green	Pale green	
(*e*) Iron hematoxylin	Undifferentiated germ cells	Blocks	Blue black	Blue black		
	Oogonia	Strands with beads	Blue black	Blue black	Blue black	
	Adult oocyte	Minute threads joining granules	Blue black	Blue black	Blue black	Blue black
	Spermatocyte	Large granules, tiny threads	Blue black	Blue black	Blue black	
(*f*) Iodine ripened hematoxylin	Undifferentiated germ cell	Blocks	Purple	Purple		
	Oogonia	Strands with beads	Purple	Purple	Purple	
	Adult oocyte	Minute threads joining granules	Purple	Purple	Purple	Purple
	Spermatocyte	Large granules, tiny threads	Purple	Purple	Purple	

(*d*) Semmen's and Bhaduri's modification of the Feulgen light green method (15, 16). According to Caspersson and Schultz (5) "the ribonucleic acids are dissolved in the carbonate solution since a similar procedure can be used for their extraction when it is desired to separate them from the thymonucleic acid. The reaction to the stain after the solution of the ribonucleic acids would then be due to the presence of highly basic proteins (protamines, histones) in the nucleolus".

(*e*) Pappenheim's methyl green pyronin G—Unna's modification (Lee (9)). This technique is "especially valuable when it is desired to distinguish between chromatin and other basophilic material in the cell, as the method is specific for chromatin". The chromatin is stained a greenish violet color and the basophilic constituents of the cell are colored red.

(*f*) Heidenhain's iron alum hematoxylin counterstained in light green (Guyer (8)).

The staining reactions of the cell constituents are summarized in Table I.

Normal Developments of Germ Cells (Fig. 1)

The germinal epithelium lining the ovotestis consists of a single layer of flattened cells, containing an elliptical nucleus, in which the chromatin appears in small clumps. When differentiation starts, the germinal epithelial cells begin to enlarge, changing from a somewhat rectangular shape to a more rounded appearance, accompanied by a rapid increase in size of the nucleus. The cells proceed from this stage to undergo development into either a spermatogonium, an oogonium, or a nurse cell. As the animals approach old age, one may sometimes observe a change in the germinal epithelium cells, which show a tendency to elongate towards the lumen of the follicle and become somewhat untidy looking. In spite of these changes in size, these cells have not been observed undergoing mitosis.

The Spermatogonia

The single follicle of a newly hatched slug is lined by epithelium and the lumen is full of spermatogonia, but during the growth of the ovotestis, the development of these male cells can be followed. The changes involve great increase in the size of the nucleus, which is now surrounded by a thin layer of cytoplasm. A prominent nucleolus, lying to one side of the nucleus, but not at its periphery, appears quite early in the development of the spermatogonium and the various staining methods indicate its oxyphilic nature. In many cases, two of these nucleoli appear, but there is no sign of a basophilic nucleolus (Fig. 7). The nucleoli may be followed up to the zygotene stage of the prophase preceding the first meiotic division but they disappear by late diplotene. The nucleolar cycle has not been followed beyond this stage, as neither secondary spermatocyte nor spermatid show the presence of a nucleolus with the methods used in this work.

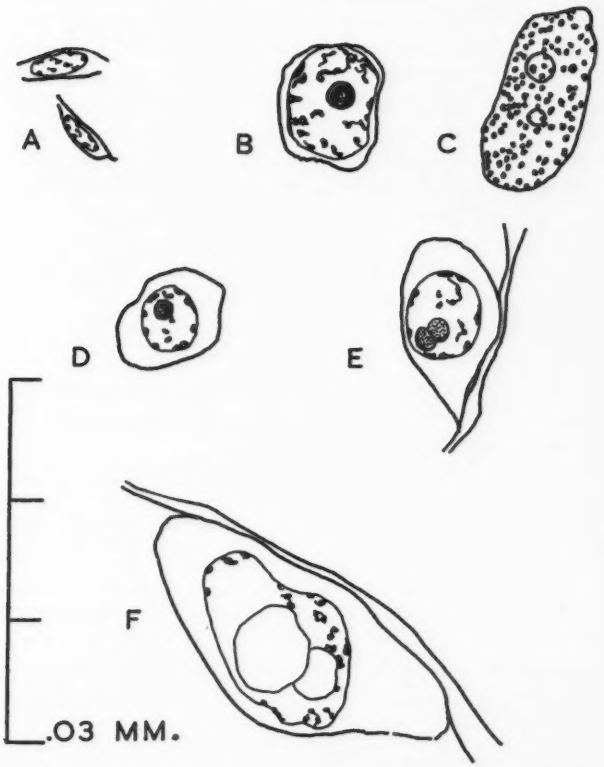


FIG. 1. *A*. Germinal epithelium from young animal. *B*. Early spermatogonium in lumen of follicle. *C*. Nucleus of nurse cell with two nucleoli. *D*. Very young oocyte with one oxyphilic nucleolus. *E*. Young oocyte with two nucleoli, both oxyphilic. *F*. Older oocyte with two oxyphilic nuceoli.

Nurse Cells

The nuclei of these cells are ovoid and large and they display the deeply-staining chromatin blocks suggestive of the polyploid cells sometimes seen in insect follicles. There are two nucleoli present in many of the nuclei, which are surrounded by indistinct cytoplasm and an extremely thin cell wall, often quite difficult to see (Fig. 8).

Oocytes

When the slugs are between 15 and 20 days old, the germinal epithelium begins to give rise to oocytes, which show a rapid increase in the volume of both nucleus and cytoplasm. The nucleus contains a single oxyphilic nucleolus and the chromatin blocks move to the periphery of the nucleus (Fig. 1, *D*, *E*, *F*). The oocyte continues to grow and at 24 days a second oxyphilic nucleolus appears equal in diameter to the first, the two being closely in contact.

The shape of the oocyte has by now changed somewhat and this is seen in Fig. 1, *E*. The characteristic feature of this oocyte is its firm attachment to the wall of the follicle, from which it will not become detached until the eggs leave the acinus to travel up the oviduct where presumably they complete their maturation division.

The changes which take place in the nucleolus during the later stages are summarized in Table II.

TABLE II

THE DEVELOPMENT OF THE NUCLEOLUS IN THE GROWING OOCYTE

Age of animal after hatching	Appearance of nucleolus
15 – 20 days	First appearance of oocyte with a single oxyphilic nucleolus
24 days	Second oxyphilic nucleolus equal in size to the first, one overlapping the other
30 days	One nucleolus is twice the size of the other
46 days	Both nucleoli are still oxyphilic
Adult animal	Large nucleolus becomes basophilic. Smaller nucleolus remains oxyphilic. Both are homogeneous in appearance, often separated from one another in the center of the nucleus (Fig. 9).
	Extrusion of oxyphilic nucleolar material into nucleoplasm.
	A third nucleolar body may appear, staining red with Mann's methyl blue eosin, like the small nucleolus.

Just before the extrusion of the nucleolar material, the large nucleolus may show a change in staining intensity in one side, resulting in a cap of deeply stained material, which does not, however, alter the spherical shape of the nucleolus, suggesting that the material has accumulated from within the nucleolus itself, rather than having been added to its surface.

Following the period of nucleolar extrusion, the nucleolus loses its homogeneous appearance, and now presents a coarsely granular texture, with a condensation of the material accumulating at the periphery of the nucleolus to form a distinct rim. Finally the nucleolar membrane appears to break down and the fragments go free in the nucleoplasm. However, these fragments seem to remain in the area formerly occupied by the intact nucleolus rather than moving out to the periphery of the nucleus. The small oxyphilic nucleolus remains homogeneous throughout and no instance of fragmentation was seen.

Experimental

A. Effects of Temperature

Animals were collected and brought into the laboratory and were kept there, at a temperature of 20° to 22° C., for several days. The slugs were divided into four groups, each of which was exposed to one of the following temperatures: −2°, 0°, 2°, and 6.5° C., for periods of time ranging from 2 to

192 hr. The animals were placed in low Petri dishes lined with moistened filter paper and fed on lettuce. These containers were placed in the compartments just below the freezing chamber of an electrical refrigerator. Temperatures of −2° and 0°C. were quite constantly maintained. A refrigeration room supplied a constant temperature 2°, and a special tank with a cooling unit kept the fourth group at 6.5° C. The animals in these two cases were kept in large glass terraria containing a layer of moistened vermiculite. These slugs withstand soil temperatures of as low as −2° C. in their normal habitat, but they do so by slow degrees and it was obvious that animals collected in the winter and the summer might react at different rates to exposure to low temperatures, since in the case of slugs living at soil temperature of 18° C. during the summer, the rapid change from a soil temperature of 18° to 0° C. would constitute a sudden shock.

Since the exposures to 0° brought about certain changes in the ovotestis more easily and regularly than at the other temperatures, only these will be recorded at the present. The animals placed in temperatures of 0° and 2° lived quite well, although they were quite inactive and ate very little. However, eggs were laid by several animals during their exposure to these temperatures.

Results

(*a*) The most obvious change in these animals exposed to low temperatures was in the form of the germinal epithelial cells. The normal thin layer of flattened cells, with elliptical nuclei as seen in Fig. 12, now appears to round out and enlarge and grow in towards the lumen of the follicle, assuming a columnar shape (Fig. 2). The nucleus becomes spherical while the volume of cytoplasm increases from 2 to 3, to 8 or 10 times its normal size.

In normal animals, increase in size of a germinal epithelium cell precedes its differentiation, but these greatly elongated cells show no such tendency. The maximum increase in these cells, shown in Fig. 14, occurred in a summer animal exposed to 0° C. for 96 hr., while an animal in winter showed a corresponding type of change after 192 hr. (Fig. 15). A comparison between a summer and winter animal exposed to 0° for 96 hr. shows that there is only a slight enlargement of the nuclei of the germinal epithelium in the latter (Fig. 13), in contrast to the greatly elongated cells already referred to. Shorter exposures of summer animals to the low temperature established the fact that growth and elongation of the germinal epithelium cells can be induced at the end of six hours, while two hours' exposure will produce an evident thickening of the epithelium without an axial change. The shortest exposure of winter animals which will bring about elongation of the cells is between 16 and 20 hr. It was thought that this effect of the low temperature for so short a period in the summer animals might prove to be temporary and that the cells would assume their original form if the animal was allowed to remain at room temperature for a corresponding period of a few hours. At the end of a few hours, no change was seen to occur in these cells and in one animal kept at room

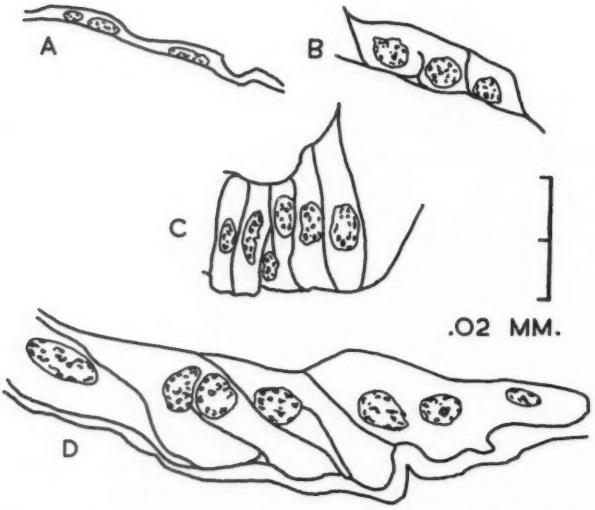


FIG. 2. Germinal epithelium in animals exposed to cold. *A*. Normal epithelium. *B*, *C*, *D*. Changes resulting from exposure of animals to 0° C. The direction of growth has changed and the cells are columnar shaped.

temperature for two months, the cells appeared to have elongated still further, and a double layer of epithelium had developed. If this irreversibility proves to be general, one might imagine that the temperature shock releases an inhibitory substance which normally regulates the form of the germinal epithelium, resulting in this greatly accelerated growth rate, with a change in the axis of the cell.

(*b*) The elongation of the epithelial cells is accompanied by other changes in the ovotestis, the most obvious of which is the premature detachment of the young eggs from the epithelial wall, and their movement into the lumen of the follicle. These eggs appear to be undergoing pathological changes in the cytoplasm and the nucleolus. The cytoplasm appears fluid in consistency at the periphery, rather than densely granular and the cell membrane is irregular (Fig. 11). In extreme cases, the cytoplasm has practically disappeared, leaving nuclei from neighboring oocytes lying close together with only a thin layer of cytoplasm on one edge (Fig. 10). The nucleoli of these cells behave differently from those in the control animals. The larger nucleolus normally shows changes in staining reactions during growth but the smaller one is rather constant in composition (Table II); the latter in these experimental animals stains violet with Mann's methyl blue eosin, which indicates that it may be undergoing a change from its original oxyphilic nature. However, these small nucleoli never stain as intensely as the larger ones. In addition to this change in staining reaction, the smaller nucleolus may show changes in shape and position. It may appear oval instead of spherical, and this change is accompanied by a rotation through 90°, so that its long axis projects away from

the larger nucleolus, before coming to rest near the periphery of the nucleus (Fig. 16). Those small nucleoli which retain their original central position frequently appear to be undergoing vacuolation, with condensation of material at the periphery, a condition which has not been observed in normal animals.

The accumulation of material on the surface of the large nucleolus in the form of a rim has been observed in the larger eggs of normal animals, but, in experimental animals, these rims appear very frequently in small as well as large oocytes (Fig. 19). In mature oocytes, the nucleolar membrane disappears and the condensed material breaks up into fragments as already described. This phenomenon appears very frequently in animals exposed to cold—in fact it is far more prevalent than the homogeneous condition of the nucleolus (Figs. 17, 18).

The effect of cold is much less marked in the male germ cells. One can find quite normal-looking stages of spermatogenesis, but the germinal epithelium in its altered form never differentiates into new spermatogonia.

Summary on Temperature Effects

These changes suggest that exposure to sudden low temperature accelerates maturity and senescence in the ovotestis by upsetting the normal mechanism of cellular control, owing to a release of an inhibiting substance, which might perhaps be an enzyme. The elongation of the germinal epithelium might be enough to cause the early separation of the eggs from the wall of the follicle; if this were the case, it could explain the marked cytoplasmic degeneration of young oocytes due to their separation from the source of nutriment normally supplied by their connection with the nurse cells.

B. The Effects of an Anterior Pituitary Hormone

The hormone which was injected into the slugs was supplied by the Parke-Davis Co. Ltd., under the name "Synapoidin". (It is a combination of chorionic gonadotrophin (luteinizing hormone) prepared from human pregnancy urine and the follicle-stimulating hormone (gonadal synergist) from the anterior pituitary, according to the information supplied with the product.)

Large animals (3 cm. in length) were injected through the posterior part of the foot, with a 1 cc. syringe and a No. 27 needle, delivering a dose of .03 cc. of the hormone into the body cavity. Smaller animals were given a slightly smaller dose. The controls were injected in the same way with distilled water.

The animals stood the injections quite well and it was possible to give them up to 11 doses. Several animals were killed every two days, so a series was built up which made it possible to compare animals which had received 1 dose, 2 doses, and so on up to 11 doses of the hormone.

Some animals were kept from 8 days up to 57 days after their last injection, to see whether the hormone continued to produce its effect or whether it would wear off after a suitable length of time. The experiments were carried out on

animals collected in both the winter and the summer, since the seasonal changes in egg production are important in estimating the possible effects of a hormone, which has a follicle-stimulating and luteinizing effect on vertebrate animals.

Results

(*a*) *General Effect on the Ovotestis and the Number of Eggs Produced*

The cytological examination of the cells showed three outstanding changes: a general lack of organization in the orientation of the germ cells in each acinus, a notable increase in the number of eggs in each follicle, and a rapid maturation of the male germ cells. These effects seem to be cumulative, as animals receiving two or three doses do not exhibit them to the same degree as animals receiving eight to nine doses of synapoidin.

Animals injected with nine doses of synapoidin, or a total of 2 cc. of the hormone, show a complete lack of organization of the cells: the eggs are frequently found in the lumen, instead of firmly attached to the follicle wall, and the spermatocytes and sperms are no longer neatly grouped together, but many are seen scattered throughout the follicle. Intermediate stages in this apparent condition of disorder occurred in animals receiving from three to eight doses of synapoidin.

In normal animals, one can find all stages of spermatogenesis occurring in the ovotestis, and this was so in animals which had been given up to seven doses of synapoidin; but with nine or more doses, mature sperm were found practically to the exclusion of all other stages. This effect is somewhat comparable to the results of Burns and Buyse (3) who found that extracts of mammalian hypophysis, injected into salamanders, produced a normal but precocious development of the male germ cells. Thus it seems that in slugs also, the hormone causes an increase in the rate of sperm development. There was no noticeable hypertrophy of the gonad as a whole.

In *D. reticulatum*, egg laying is continuous throughout the year, reaching a peak in June and another in September. In the summer months the average number of eggs per follicle was 1.43 for a series of 100 follicles. In the winter it dropped to 1.1 eggs per follicle. For this reason the experimental results obtained in the winter months were not averaged with those of the summer, but were plotted separately. In both series of experiments it was found that there was a gradual increase in the number of eggs per follicle, in proportion to the number of hormone injections which were given. The greater the number of injections, the greater the number of eggs observed (Fig. 3).

It was also found that the longer the interval between the last injection and the time of killing, the greater the number of eggs in the follicle, suggesting that the hormone may exert an effect for some time. As was expected, the summer animals which were injected contained a larger number of eggs than the winter group. In each case, however, the rate of rise seems to be about equal so that by the time the animal has received nine doses of the hormone, the number of eggs per follicle has doubled that found in the normal animals.

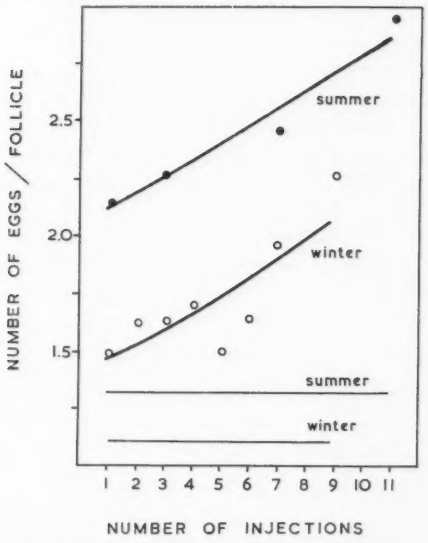


FIG. 3. Graph showing increase in number of eggs with increasing number of injections of synapoidin. The two horizontal lines represent the number of eggs found in control animals in summer and winter.

Each point on this graph represents the average number of eggs calculated for 100 follicles and it has been plotted arithmetically to show the steady rise produced by each additional injection. The curves have been calculated statistically so that they represent the best values for these scattered points. It will be noticed that in the winter animals, the calculated data do not go beyond seven injections, since there were not sufficient numbers of animals available.

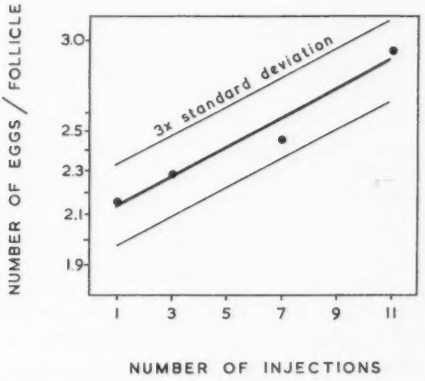


FIG. 4. Number of eggs per follicle in animals injected with synapoidin in the summer.

The correlation coefficient for the data of the summer and winter egg production in injected animals was computed and it was found that the relationship shown between the number of eggs per follicle and the number of injections represented by a straight line was valid. It can be seen from the figure that all the points lie within the range $\pm 3\sigma$ for the summer animals (Fig. 4). It was more difficult to get uniform animals in sufficiently large numbers in the winter, to give results as clear-cut as those obtained in the summer, but the graph (Fig. 5) shows that there are enough points falling within the limits of $\pm 3 \times$ standard deviation to satisfy expectations. It is evident from these two graphs that there is a significant rise in the number of eggs per follicle after injection with synapoidin and that the effect of the hormone is cumulative.

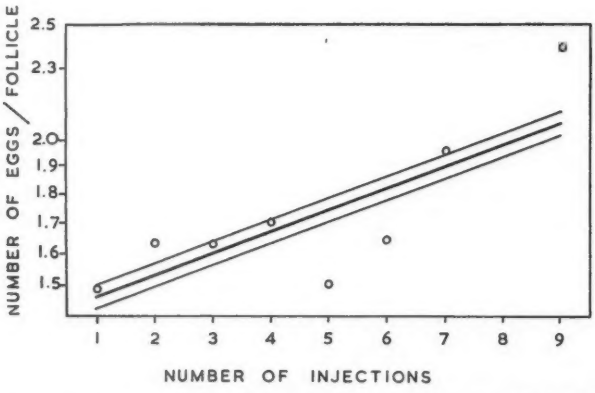


FIG. 5. Number of eggs per follicle in animals injected with synapoidin in the winter.

(*b*) *Specific Effect on the Cells*

In addition to the general disorganization of the ovotestis and the increased number of eggs produced, following injection of the hormone, the cells themselves showed various abnormalities. The eggs moved into the lumen of the follicle, even when quite small, instead of being attached to the wall of the follicle in the usual way. These small eggs often show marked changes in the cytoplasm, which may result in the egg membrane becoming irregular in outline or in the disappearance of most of the cytoplasm, so that the nucleus is lying surrounded by spermatocytes and mature sperm, instead of by the egg cytoplasm. This extreme stage of degeneration occurs after six or more doses of synapoidin, whereas smaller doses may result in no more than a noticeable vacuolization of the cytoplasm of the intact oocyte.

The nucleoli of the injected animals show many changes from the normal and Table III gives a comparison of the behavior of the nucleolus in control and experimental animals, while the appearance of some of the nucleoli of the experimental animals is illustrated in Fig. 6.

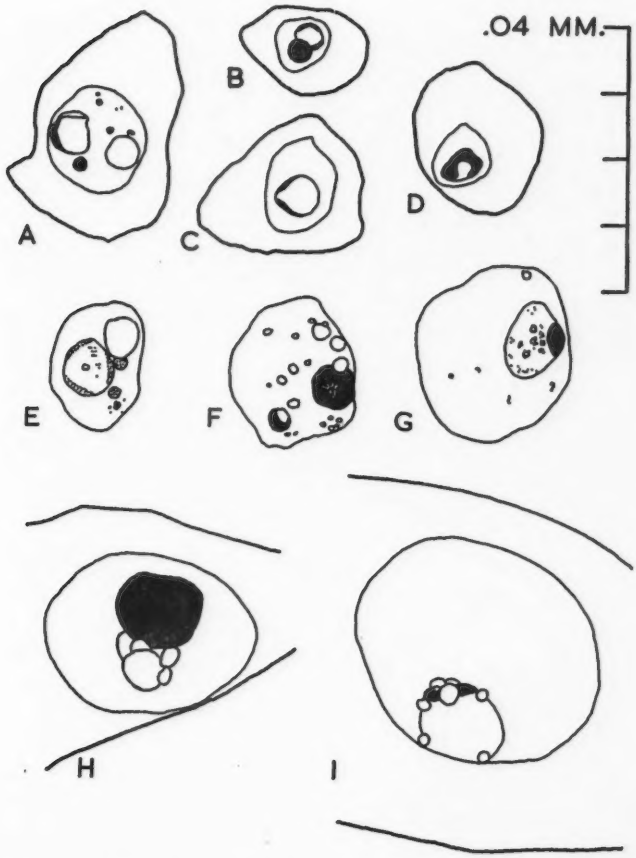


FIG. 6. Oocytes of slugs injected with synapoidin. *A, B, C, D.* Eggs with the larger nucleoli showing rims (iron hematoxylin). *E, F, G.* Nuclei from eggs which have lost their cytoplasm (Mann's methyl blue eosin). *H, I.* Eggs showing numerous oxyphilic spheres in the nuclei (Mann's methyl blue eosin).

It was thought that this type of hormone might have caused proliferation of the germinal epithelium, since it is supposed to stimulate the interstitial cells of the vertebrate gonad. However, the action of vertebrate hormones on invertebrates is as unpredictable as the reverse combination. The case of vertebrate hormones injected into distantly related vertebrate species is also difficult to analyze according to Pickford and Thompson (13) who say: "This type of experiment is subject to the difficulty that the anterior lobe hormones are proteins and, in consequence, may be ineffective when injected into distantly related species, owing either to antihormone reactions or to

TABLE III

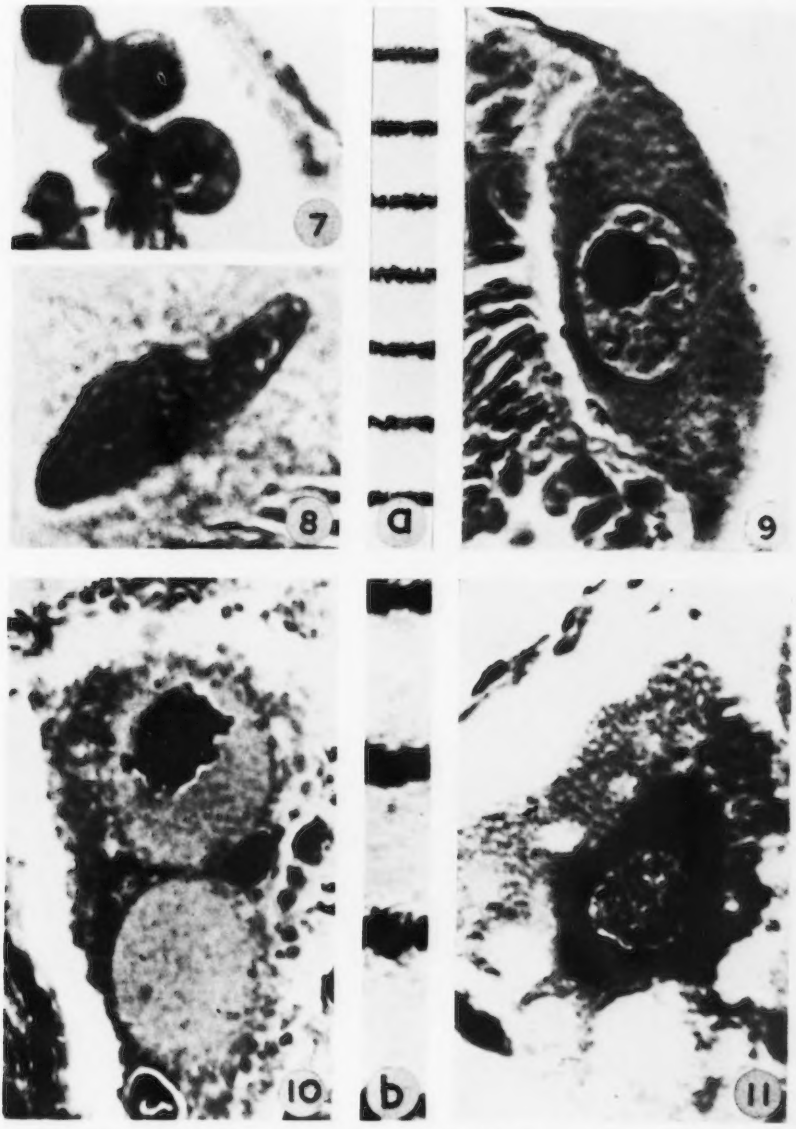
SUMMARY OF CHANGES IN THE NUCLEOLUS OF NORMAL AND EXPERIMENTAL ANIMALS

Organelle	Normal	Cold	Synapoidin
Small nucleolus	Oxyphilic (red with Mann's stain)	Partially basophilic (purple with Mann's)	Completely basophilic (blue stain)
Small nucleolus	Spherical, never peripheral in position	Oval, rotating to periphery of nucleus	Spherical never peripheral
Large nucleolus	Slightly vacuolated	Highly vacuolated	Highly vacuolated (Fig. 6, *E*)
Large nucleolus	Basophilic material occasionally forms deeply-staining rim, leaving a light center in older oocytes	Rims occur frequently in small and large oocytes	Many rims seen in small and large oocytes (Fig. 6, *A*, *B*, *C*, *D*)
Large nucleolus	In older oocytes nucleolar membrane breaks down, releasing basophilic material into nucleus	Fragmentation occurs much more frequently in eggs of various sizes	Fragmentation more frequent
Large nuceolus	Tiny oxyphilic spheres are released into nucleoplasm, prior to breakdown of nucleolar membrane	Oxyphilic and basophilic spheres are released into nucleus	Extrusion of oxyphilic spheres much more frequently seen and more intense, especially where the cytoplasm has broken down. Spheres also occur with basophilic rims surrounding a colorless center (Fig. 6 *E*, *F*, *G*, *H*, I)

other manifestations of protein specifications. Thus, negative results might be meaningless but positive results, even if not very pronounced, must yield strong a priori evidence".

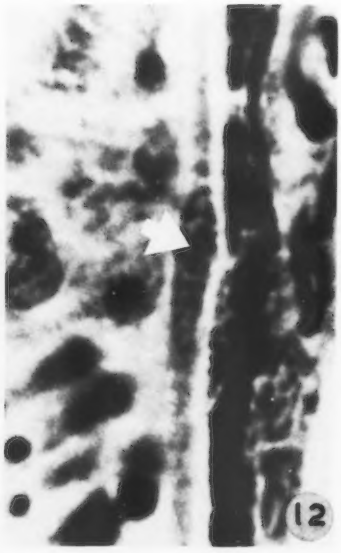
Discussion

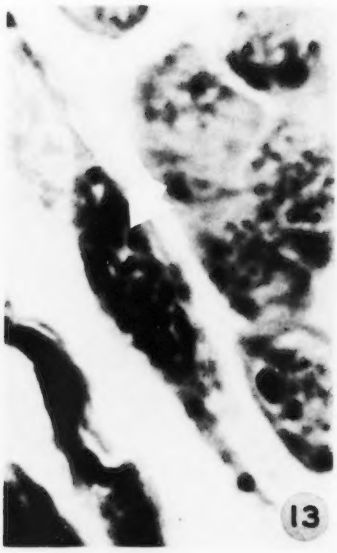
The use of low temperatures and hormones as agents for inducing cellular changes in the ovotestes of slugs results in a similar type of general disorganization of the gonad, which is practically indistinguishable in the two kinds of treated animals, and this similarity extends to the behavior of the nucleoli which is shown in Table III. That two seemingly unrelated factors such as cold and a hormone exert their influence on this organelle suggests that the close relationship between cytoplasmic protein synthesis and the nucleolus visualized by Caspersson (4) is broken down resulting in the degeneration of the egg, with entire loss of the cytoplasm in extreme cases. It is noticeable

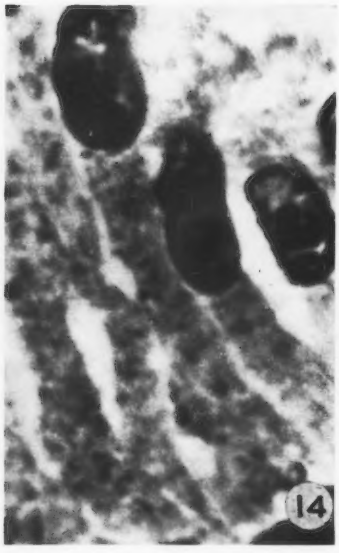


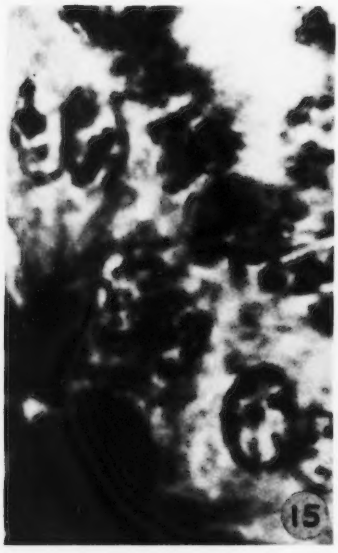
FIGS. 7–11. FIG. 7. Normal spermatogonial cell with two nucleoli. FIG. 8. Normal nurse cell with two nucleoli. FIG. 9. Normal egg showing the large basophilic and the small oxyphilic nucleolus. FIG. 10. Two eggs from animal exposed to 0° C. for 16 hr., showing breaking down of cytoplasm and the nuclei resting side by side. FIG. 11. Egg from animal exposed to 0° C. for four hours, showing irregular outline of cytoplasm. Scales a and b show magnification at which photographs were made. One division equals 0.01 mm.

FIGS. 7, 9, 10, 11 were photographed at the magnification of scale a. All other figures on Plates I, II, and III have the same magnification as scale b.

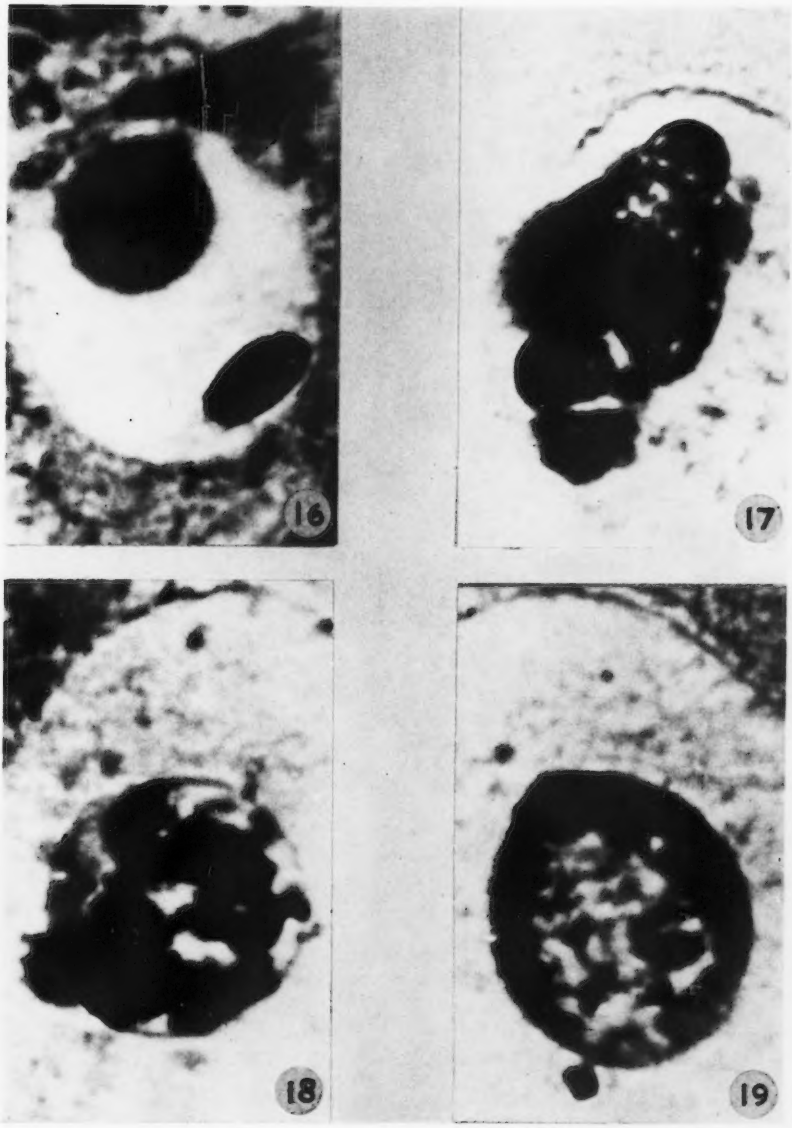








FIGS. 12–15. FIG. 12. Normal germinal epithelial cell, indicated by arrow, showing prominent nucleus with chromatin in form of blocks. FIG. 13. Germinal epithelial cell, indicated by arrow, from animal exposed to 0° C. in winter for 96 hr. Note slight enlargement of nucleus. FIG. 14. Germinal epithelial cells from animal exposed to 0° C. in summer for 96 hr. Note elongation of cells and rounding out of nuclei. FIG. 15. Germinal epithelial cells from animal exposed to 0° C. in winter for 192 hr. Cells are elongated and nuclei are rounding out.



FIGS. 16–19. FIG. 16. Egg from animal exposed to 2° C. for 24 hr. showing smaller nucleolus oval in shape and at the periphery. FIG. 17. Egg from animal exposed to 0° C. for six hours showing large nucleolus with several spheres, both oxyphilic and basophilic, bubbling out. FIG. 18. Egg from animal exposed to 0° C. for 120 hr. showing condensation of material in larger nucleolus. FIG. 19. Egg from animal exposed to 0° C. for 120 hr. showing condensation and fragmentation of large nucleolus, a condition sometimes seen in control animals.

that the nucleus itself is far less sensitive to these agents and never loses its identity. The accelerated growth and maturation of the germ cells which follows the injection of a vertebrate hormone suggests that their normal development may also be under the control of a hormonal system which must initially direct the course followed by the germinal epithelium. Obviously the animals were unable to adjust themselves to this precocious onset of sexual maturity, since the death rate increased greatly with increased doses of the hormone.

The most striking difference between the effects of these two agents is seen in the behavior of the germinal epithelium. After low temperature treatment, the cells change their direction of growth and show a marked increase in volume, whereas they show no such change following treatment with synapoidin. The effect of the cold may be compared to an induced senescence, since old animals under normal conditions show a slight tendency to elongation and thickening of the epithelial cells.

We are indebted to the National Cancer Institute for a grant while this work was being carried out.

References

1. ANCEL, P. Histogénèse et structure de la glande hermaphrodite d'*Helix pomatia*. Arch. biol. (Liège), 19 : 389–652. 1903.
2. BAKER, J. R. The structure and chemical composition of the Golgi element. Quart. J. Microscop. Sci. 85 : 1–71. 1944.
3. BURNS, R. K. and BUYSE, A. Effect of mammalian hypophyses on the reproductive system of immature male salamanders. J. Exptl. Zoöl. 67 : 67–115. 1934.
4. CASPERSSON, T. O. Cell growth and cell function. A cytochemical study. W. W. Norton & Company, Inc., New York. 1950.
5. CASPERSSON, T. O. and SCHULTZ, J. Ribonucleic acids in both nucleus and cytoplasm and function of nucleolus. Proc. Natl. Acad. Sci. U.S. 26 : 507–515. 1940.
6. DE TOMASI, J. A. Improving technic of the Feulgen stain. Stain Technol. 11 : 137–144. 1936.
7. GATENBY, J. B. The cytoplasmic inclusions of the germ cells (Part 5). Quart. J. Microscop Sci. 63 : 445–491. 1919.
8. GUYER, M. F. Animal micrology. The University of Chicago Press, Chicago. 1947.
9. LEE, B. The microtomists' vade mecum. 10th ed. P. Blakiston's Son & Co., Inc., Philadelphia. 1937.
10. LUDFORD, R. J. The behaviour of the nucleolus during oogenesis, with special reference to the mollusc *Patella*. J. Roy. Microscop. Soc. 62 : 121–133. 1921.
11. ORD, M. J. and WATTS, A. H. G. New records for the distribution of certain land molluscs in Nova Scotia. Proc. Nova Scotian Inst. Sci. 22 : 16–35. 1948.
12. PANTIN, C. F. A. Microscopical technique. Cambridge University Press., London. 1946.
13. PICKFORD, G. E. and THOMPSON, E. F. The effect of purified mammalian growth hormone on the killifish (Fundulus). J. Exptl. Zoöl. 109 : 367–383. 1948.
14. PILSBRY, H. A. Land Mollusca of North America. Acad. Nat. Sci. Phila. 2 (2) : 533. 1948.
15. SEMMENS, C. S. and BHADURI, P. N. A technique for differential staining of the nucleoli and chromosomes. Stain Technol. 14 : 1–5. 1939.
16. SEMMENS, C. S. and BHADURI, P. N. Staining of the nucleolus. Stain Technol. 16 : 119–120. 1941.

A NOTE ON THE CHIGGERS (TROMBICULIDAE) OF ALBERTA[1]

By John H. Brown[2] and James M. Brennan[3]

Abstract

Seven species of chiggers are reported from Alberta, six of these for the first time. All species are from small rodents and three are reported from *Citellus richardsonii*, the common ground squirrel of the Alberta prairies. *Euschöngastia campi* n. sp. is described and figured. All were collected by field crews of the Division of Entomology, Alberta Department of Public Health, and were determined by the junior author.

Introduction

With the exception of a few published records, almost no information is available on Canadian chiggers. Recently, the collecting of ectoparasites by field crews of the Division of Entomology, Alberta Department of Public Health, in the course of spotted fever and plague surveys in Alberta has afforded an opportunity to acquire some knowledge of the chiggers in this Province. Seven species along with host and distributional data are recorded. Of these, *Trombicula microti* Ewing, has been reported previously by Brennan and Wharton (1) and *Euschöngastia campi*, a new species, is described herein.

Euschöngastia campi n. sp.

(Fig. 1, Fig. 2)

Body: Length and width of holotype, engorged, 608 by 495 μ. Striae conspicuous, fine. Eyes 2/2, large, the anterior and posterior well separated. Anus at level of fifth row of ventral setae.

Puncta: The series on which this species is based is comprised of 87 well engorged specimens representing six different collections. Puncta could not be discerned in any individual on any of the usual puncta-bearing structures, namely, the palpal femora, capitular sternum, cheliceral bases, scutum, and leg segments including the coxae.

Gnathosoma: Chelicerae quite small, the blades with tricuspid caps. All palpal setae branched except the nude lateral tibial. Palpal claw with five conspicuous prongs. Palpal tarsus with six branched setae and a fairly long spur. Galeal seta branched.

Scutum: at least three times broader than long, shaped as figured (Fig. 1, *B*). Scutal setae heavily barbed, the posterolaterals much longer than the anterolaterals. Sensillae fusiform and with numerous strong setules which continue

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Contribution from the Division of Entomology, Alberta Department of Public Health, Edmonton, Alta. Contribution No. 46.

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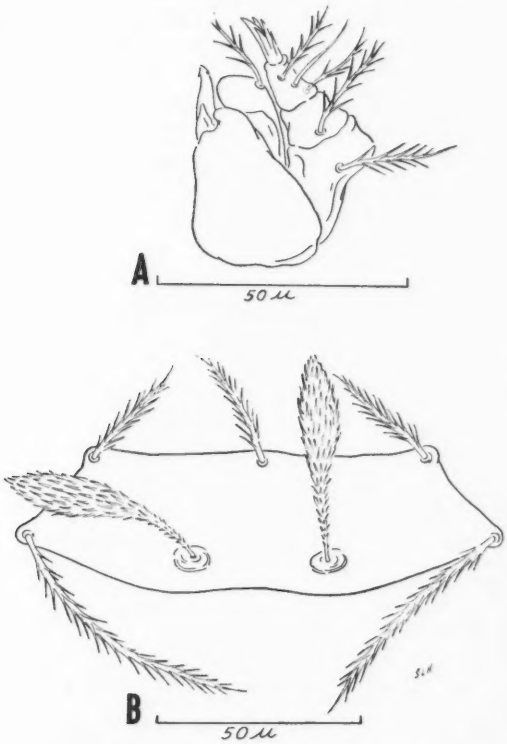

FIG. 1. *Euschöngastia campi* n. sp.
A. Dorsal view of right half of gnathosoma, palpal tarsus omitted; B. scutum.

nearly to the bases. Sensillary bases posterior to the level of the posterolateral setae and very close to the posterior margin of the scutum. Scutal measurements of holotype: AW-74, PW-112, SB-35, ASB-26, PSB-11, AP-24, AM-39, AL-32, PL-64, S-52.

Legs: Setae distributed as follows: Leg I coxa, trochanter, and basifemur each with a branched seta; telofemur with five branched setae; genu with four branched setae, two very long genualae and a microgenuala; tibia with eight branched setae, two long tibialae, and a microtibiala; tarsus with about 16 branched setae, a spur, a microspur, a very short parasubterminala, a subterminala, and a pretarsala. Leg II coxa and trochanter each with a branched seta; basifemur with two branched setae; telofemur with four branched setae; genu with three branched setae and a long genuala; tibia with six branched setae and two tibialae; tarsus with about 14 branched setae; a spur, a microspur, and a pretarsala. Leg III coxa and trochanter each with a branched seta; basifemur with two branched setae; telofemur with three branched setae;

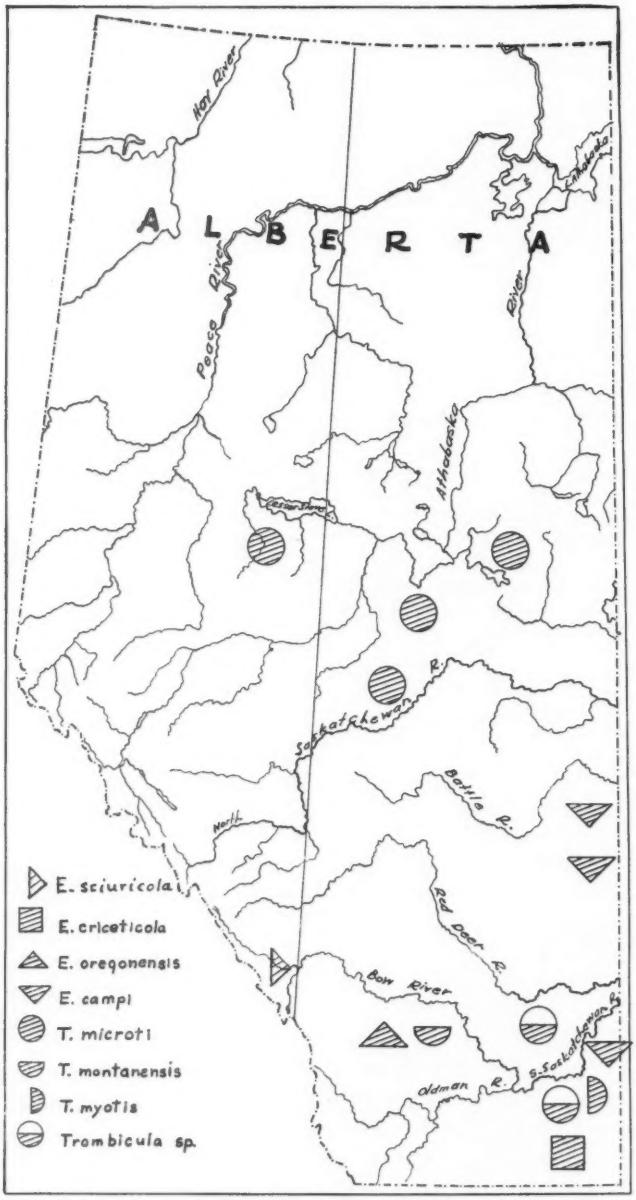

FIG. 2. Distribution.

Euschöngastia sciuricola
E. criceticola
E. oregonensis
E. campi
Trombicula microti
T. montanensis
T. myotis
Trombicula sp.

genu with three branched setae and a genuala; tibia with six branched setae; tarsus with about 12 branched setae. All tarsi terminated by two claws and an empodium.

Setae: Dorsal setae long, slender, similar to the scutal setae in form and decreasing slightly in length from anterior to posterior rows. Rows somewhat irregular. Dorsal formula for the holotype: 2–12–10–8–6–2–2. In other specimens the dorsal formula may begin: 2–10–10 . . . . . , 2–11–12 . . . . . , etc. Ventral setae 2–2 (sternals) plus about 28.

Type data: Holotype and 38 paratypes, R.M.L. No. 28556, from Richardson ground squirrel, *Citellus richardsonii*, Sibbald, Alta, 9 July 1950; 11 paratypes, R.M.L. No. 28555, same host and locality, 7 July 1950; 4 paratypes, R.M.L. No. 28557, same host and locality, 10 July 1950; 6 paratypes, R.M.L. No. 28558, and 26 paratypes, R.M.L. No. 28559, same host, Compeer, Alta., 17 July 1950; 1 paratype, R.M.L. No. 29130, same host, Irvine, Alta., 30 June 1951. All collections by personnel of the Alberta Department of Public Health, Division of Entomology.

Holotype and some paratypes in the collection of the Rocky Mountain Laboratory. Other paratypes distributed to the United States National Museum, the British Museum (Natural History), the South Australian Museum at Adelaide, the University of Kansas, the collection of Charles D. Radford, and the Canadian National Collection, Ottawa.

Diagnosis: Euschöngastia campi n. sp. is related to *E. blarinae* (Ewing) and *E. sciuricola* (Ewing) by virtue of the five-pronged palpal claw, AL much shorter than PL, and tibia III lacking a tibiala, but is readily distinguished from the former by the longer and more slender sensillae and the impunctate scutum and leg segments, and from the latter by the fusiform sensillae.

The other species known to be present in Alberta are recorded below with their host animals, locations (also see Fig. 2), and dates collected.

Host	Location	Date Collected
1. *Euschöngastia criceticola* Brennan		
Peromyscus maniculatus osgoodi	Manyberries	May 12, 1951
2. *Euschöngastia oregonensis* (Ewing)		
Citellus richardsonii	Lomond	June 24, 1950
C. richardsonii	Lomond	June 25, 1950
3. *Euschöngastia sciuricola* (Ewing)		
Eutamias amoenus	Lake Louise	June 24, 1952
E. amoenus	Banff National Park (Saskatchewan River Crossing)	June 20, 1952
Phenacomys intermedius	Banff–Louise Highway (Mile 30)	June 24, 1952

Host	Location	Date Collected
4. *Trombicula microti* Ewing. This species is probably the common chigger in Alberta.		
Clethrionomys gapperi	Edmonton	Sept. 2, 1948
Eutamias amoenus	Edmonton	Sept. 2, 1948
Peromyscus maniculatus	Edmonton	Sept. 2, 1948
Microtus sp.	Edmonton	Sept. 15, 1949
P. maniculatus	Lac la Biche	Aug. 17, 1950
C. gapperi	Lac la Biche	Aug. 17, 1950
C. gapperi	Athabasca	Aug. 22, 1950
C. gapperi	Faust	Aug. 30, 1950
P. maniculatus	Faust	Aug. 30, 1950
5. *Trombicula montanensis* Brennan		
Citellus richardsonii	Lomond	June 24, 1950
C. richardsonii	Lomond	June 25, 1950
Mus musculus	Lomond	June 27, 1950
6. *Trombicula myotis* Ewing. This species is characteristically a bat parasite. While the specimens recorded here are not quite typical, they can not be separated specifically from the Ewing species.		
Peromyscus leucopus	Elkwater (Cypress Hills)	July 20, 1948
7. *Trombicula* sp. (Specimens indeterminate because of poor condition.)		
Citellus richardsonii	Brooks	June 23, 1951
C. richardsonii	Irvine	June 30, 1951

The following list records the species of chiggers according to host animals.

Host species	Chigger species
1. *Citellus richardsonii*	*Trombicula* sp
	Euschöngastia oregonensis
	E. campi
	T. montanensis
2. *Peromyscus maniculatus osgoodi*	*E. criceticola*
3. *Peromyscus maniculatus*	*T. microti*
4. *Clethrionomys gapperi*	*T. microti*
5. *Eutamias amoenus*	*T. microti*
	E. sciuricola
6. *Microtus* sp.	*T. microti*
7. *Mus musculus*	*T. montanensis*
8. *Peromyscus leucopus*	*T. myotis*
9. *Phenacomys intermedius*	*E. sciuricola*

Acknowledgments

Thanks are extended to A. E. Sovereign, C. E. Collins, J. R. Higgin, and Sterling Haynes for their interest in collecting the material, and to the Misses E. Olsen and P. Wincentaylo for the care with which they sorted the pooled collections of ectoparasites. Dr. J. E. Moore, Department of Zoology, University of Alberta, collected the specimens of *E. sciuricola*.

Reference

1. BRENNAN, JAMES M. and WHARTON, G. W. Studies on North American chiggers. No. 3. The subgenus *Neotrombicula*. Am. Midland Naturalist, 44 (1) : 153–197. 1950.

DIROFILARIA DESPORTESI SP. NOV., A FILARIID FROM THE BLACK BEAR IN CANADA[1]

By LAURENT P. E. CHOQUETTE[2]

Abstract

Dirofilaria desportesi sp. nov. (Family Filariidae, subfamily Filariinae) is described from the black bear (*Euarctos americanus americanus*).

Introduction

Of the various species ascribed to the nematode genus *Dirofilaria* Railliet and Henry, 1911, only one has been reported from bears, namely, *D. ursi* Yamaguti, 1941 (29), from *Ursus torquatus japonicus* Sclegel., in Japan. Anderson (1) demonstrated, but did not describe, a species of *Dirofilaria* from the North American black bear *(Euarctos a. americanus)*. Recently, the writer had the opportunity to examine specimens of *Dirofilaria* from the submucosa of the oesophagus of a black bear from the vicinity of Timmins, Ont., and identical material collected in 1937 and 1948 from the abdominal cavity of bears from the Laurentide Park and Lake Edward, Quebec. This material consists of several worms of both sexes. Examination of the female worms showed them to be so similar to the form described by Yamaguti as to make it difficult to judge whether one was dealing with identical or different species. However, as Yamaguti's description was based upon the study of three female specimens only, it is felt that until the male of the form described by the Japanese worker is known, the present material should be considered as belonging to a different species. This is in conformity with the fact that it is better to have a species known under two different names than two species under a single name. The writer's species is described and named after the late Dr. Camille Desportes of the Institut de Parasitologie, Faculté de Médecine, Paris.

Description

The cuticle is finely striated on the dorsal and ventral surfaces and the body tapers and ends bluntly at both extremities. The cephalic end (Fig. 1) bears 10 papillae: 1 pair of lateral amphids placed about 40μ from the lateral margin of the mouth, 2 pairs submedian on each side of the head, 1 pair dorsal and 1 pair ventral to the mouth opening. The cervical papillae, not easily seen, are represented by cuticular depressions into which opens a very small canal. Such pupillae were well described by Desportes (9) in his

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Contribution from the Institute of Parasitology, McGill University, Macdonald College, P.Q., with financial assistance from the National Research Council of Canada.

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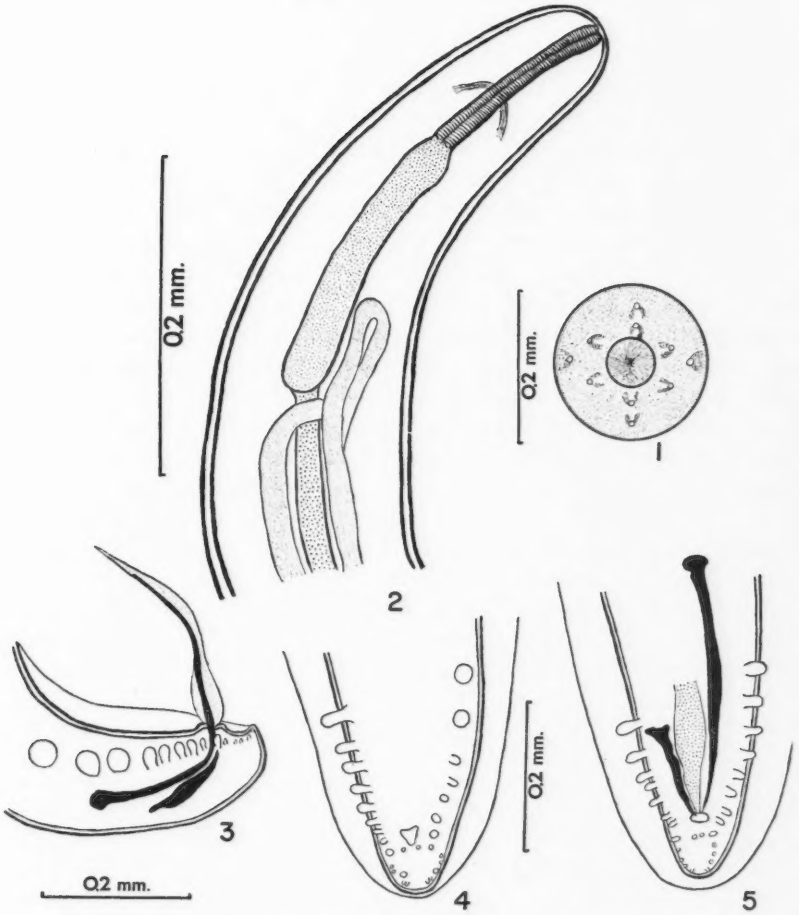

Figs. 1-5. *Dirofilaria desportesi.*
Fig. 1. Cephalic extremity, *en face* view.
Fig. 2. Anterior extremity of female.
Figs. 3, 4, 5. Posterior extremity of male, ventral views.

study of *D. conjunctivae.* In a few female specimens in which they could be seen, they were at about 153μ from the anterior end. The oesophagus (Fig. 2) is divided into two portions: the anterior part muscular, the posterior part glandular.

The female worms range in size from 188 to 231 mm. long. The anterior portion of the oesophagus is 0.46 to 0.63 mm. long and the posterior part,

0.70 to 0.96 mm. long. The nerve ring, placed on the anterior portion of the oesophagus, is 0.30 to 0.43 mm. from the anterior extremity. The width of the worm at this level is 0.30 to 0.40 mm. The vulva is 2.10 to 2.88 mm. from the cephalic extremity. The vagina may proceed posteriorly, as is the case in some specimens, or, it may form anterior loops reaching almost to the middle of the glandular portion of the oesophagus. The embryos, coiled on themselves, are about 0.25 mm. long. The convoluted ovaries extend posteriorly less than 1 mm. from the posterior end and the anus is 0.18 to 0.22 mm. from this extremity.

The male worm ranges in size from 79 to 85 mm. in length with a width, at the level of the nerve ring, of 0.25 to 0.29 mm. The anterior portion of the oesophagus is 0.40 to 0.49 mm. long and the posterior portion is 0.67 to 0.82 mm. long. The nerve ring is 0.28 to 0.31 mm. from the anterior end. The spicules are dissimilar. The right, short spicule is 0.15 to 0.17 mm. long; the left one, alated, is 0.35 to 0.50 mm. long. In one specimen in which the long spicule was evaginated, the alae could be seen for a length of 36μ past the pointed, distal extremity. The posterior end is usually loosely coiled and the anus is about 0.75 from its tip. The caudal papillae (Figs. 3-5) consist of a preanal group of pedunculated papillae, seven on one side and eight on the other, a pair of adanal and six pairs of postanal papillae, five of which are placed in single file extending to the tip of the tail, with a smaller pair between the first pair. The second and third pairs are usually quite small and, in a few specimens, the most posterior pair is double.

Discussion

As mentioned above, the female of *D. desportesi* closely resembles *D. ursi* Yamaguti, 1941, although in the former the vaginal loops do not extend as far anteriorly as they do in the latter. However, this character appears to be quite variable as in some of the mature females examined no such loops were found. On the other hand, the small caudal papillae observed by Yamaguti were not seen in any of the females examined.

As shown in Table I, *D. desportesi* differs from many of the species ascribed to this genus either in the number and arrangement of the caudal papillae or in the size and ratio of the spicules of the male. Morphological data show that the male of *D. desportesi* resembles closely that of *D. corynodes* and *D. schoutedeni* described from species of African monkeys. However, the present species differs from the simian forms in the arrangement of the caudal papillae, particularly in the postanal group. Chitwood (8), after a study of material from a Canadian lynx, concluded that *D. acutiscula* was identical with *D. repens*, but, as pointed out by Chandler (7), Chitwood's view has not been accepted by other students of the genus. *D. desportesi* also differs from

TABLE I

THE SIZE AND RATIO OF SPICULES AND NUMBER AND ARRANGEMENT OF THE CAUDAL PAPILLAE IN MALES OF *Dirofilaria*

Species	Spicules (mm.)		Ratio	Caudal papillae	References
	Long	Short			
acutiuscula (Molin, 1858)	0.346–0.616	0.130–0.240	2.5–2.6 : 1	4–7 pairs preanal; 3 pairs adanal; number and arrangement variable in postanal group	Lent and Freitas (16)
asymetrica Kreis, 1938	0.266–0.274	0.144–0.161	1.7–1.8 : 1	5–6 pairs preanal; 1 pair adanal; 1 pair postanal	Kreis (15)
corynodes (Linstow, 1899)	0.480	0.180	2.7 : 1	8 pairs preanal; 6 pairs postanal	Linstow, von (17)
freitasi Mendonça, 1948	0.260–0.330	0.220–0.240	1.2–1.4 : 1	4 pairs preanal; 4 pairs postanal	Mendonça (20)
genettae Baylis, 1928	0.490	0.180–0.190	2.6 : 1	Preanal papillae; 4 pairs sublateral; postanal: 3 pairs sublateral and 3 pairs subventral	Baylis (2)
granulosa (Linstow, 1906)	0.520	0.130	4 : 1	4 pairs preanal; 2 pairs postanal	Linstow, von (18)
immitis (Leidy, 1856)	0.300–0.355	0.200–0.226	1.5 : 1	3–4 pairs large, asymmetrical preanal; postanal: 1 pair large, smooth, asymmetrical and 3–4 pairs smooth, very small	Faust *et al.* (12)
incrassata (Molin, 1858)	0.426–0.437	0.114–0.125	3.4–3.7 : 1	3 pairs preanal; 1 pair adanal; 2 pairs postanal	Caballero (3)
	0.550	0.130	4 : 1	3 pairs preanal; 1 pair paranal; 2–3 pairs postanal	Sandground (24)

TABLE I

THE SIZE AND RATIO OF SPICULES AND NUMBER AND ARRANGEMENT OF THE CAUDAL PAPILLAE IN MALES OF *Dirofilaria*
—*Continued*

Species	Spicules (mm.)		Ratio	Caudal papillae	References
	Long	Short			
indica Chakravarty, 1936	0.385	0.165	2.3 : 1	Preanal: 4 pairs large, "granular," bluntly digitate and one small papillae, median ventral; adanal: 1 pair slender; postanal: 9 pairs, some pedunculate, some sessile, varying in size but smaller than preanal	Faust *et al.* (12)
kuelzi (Rodenwaldt, 1910)	0.210	0.125	1.7 : 1	5 pairs preanal; 2 pairs postanal	Rodenwaldt (22)
louisianensis Faust *et al.*, 1941	0.196	0.150	1.3 : 1	Preanal: 4 pairs large, smooth, symmetrical; postanal: 4 pairs, smooth, symmetrical with first and last pair smaller	Faust *et al.* (12)
macacae Sandground, 1933	0.410	0.150–0.180	2.3 : 1	5–6 pairs preanal	Sandground (23)
magalhaesi (Blanchard, 1896)	Apparently single, 0.17. Shorter, 0.23; longer undoubtedly present			Preanal: 4 pairs, large, "villous," symmetrical; postanal: 4 pairs "villous," symmetrical with last 2 pairs smaller	Faust *et al.* (12)
minor Sandground, 1935	0.280–0.350	0.135–0.175	2 : 1	4–6 and 6–4 pairs preanal papillae; 4–5 pairs postanal	Sandground (23)
nasuae Mazza, 1926	0.320	0.200	1.6 : 1	4 pairs preanal; 1 pair postanal	Mazza (19)
pagunae Sandground, 1926	0.252	0.146	1.7 : 1	4–5 pairs preanal	Sandground (23)
pongoi Vogel and Vogelsang, 1930	0.306–0.312	0.180–0.195	1.6–1.7 : 1	Preanal: 4 pairs, large club-shaped having appearance of "pine cones"; postanal: 3–4 pairs, small, digitate to conical with 1 pair very small, close together and immediately postanal	Vogel and Vogelsang (27)

TABLE I

THE SIZE AND RATIO OF SPICULES AND NUMBER AND ARRANGEMENT OF THE CAUDAL PAPILLAE IN MALES OF *Dirofilaria*
—Concluded

Species	Spicules (mm.)		Ratio	Caudal papillae	References
	Long	Short			
repens Railliet and Henry, 1911	0.465–0.590	0.185–0.206	2.5–2.8 : 1	Preanal: asymmetrical, 2–4 pairs on one side, 5–6 on the other of which one pair is adanal or slightly postanal	Canavan (4, 5)
(= *D. conjunctivae* (Addario, 1885))					Desportes (10)
scapiceps (Leidy, 1886)	0.115–0.135	0.85	1.4 : 1	Preanal: 3 pairs; postanal: 2 pairs Also, 2 pairs of sessile papillae, one pair preanal and one pair postanal near the cloacal aperture	Hall (14)
schoutedeni Vuylsteke and Rodhain, 1938	0.448	0.110	4 : 1	4–6 preanal; postanal, 5 pairs plus 1 pair immediately behind cloaca	Vuylsteke *et al.* (28)
spectans Freitas and Lent, 1949	0.350–0.400	0.180–0.210	2 : 1	Preanal: 3 pairs; 1 pair adanal lateral; 6 pairs postanal	Freitas and Lent (13)
spinosa Canavan, 1929	0.300	0.105	3 : 1	Asymmetrical with 4 pairs on right side (preanal), 9 pairs on left side of which 1 adanal and 1 postanal	Canavan (4, 5)
spirocauda (Linstow, 1858) (sea lion)	0.316–0.330	0.210–0.220	1.5 : 1	Preanal: 3–4 pairs, semisessile; 2 pairs of minute papillae lateral to the cloaca; postanal: 3 pairs of which one is a larger one	Faust (11)
subcutanea (Linstow, 1899)	0.440	0.110	4 : 1	Preanal: 6–7 pairs; postanal, 6 pairs	Linstow, von (17)
sudanensis (Linstow in Shipley, 1902)	0.270	0.230	1–2 : 1	Preanal: 6 pairs; paranal: 1 pair; postanal: 3 pairs	Shipley (25)
sudermata (Mönnig, 1924)	0.680	0.180	3.1 : 1	3–4 pairs preanal; postanal: maximum 8 pairs	Mönnig (21)

D. (?) *digitata* Chandler, 1929 (6) in the morphology and absence of papillae on the tail of the female; from *D. bonnei* Vogel and Vogelsang, 1930 (27), *D. tenuis* Chandler, 1930 (7), and *D. striata* (Molin, 1858) (Van Thiel (26)) in the position of the vulva.

Host: Euarctos americanus americanus.

Location: Peritoneal cavity and submucosa of oesophagus.

Type Locality: Lake Edward, P.Q., Canada.

Holotype (male) and homeotype (female) and paratypes:

At present in the collection of the Institute of Parasitology, Macdonald College, McGill University.

Acknowledgment

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References

1. ANDERSON, R. C. A species of *Dirofilaria* from the black bear (*Euarctos a. americanus*). Program and Abstracts of the 26th Annual Meeting, American Society of Parasitologists, Chicago, Illinois, November, 1951.
2. BAYLIS, H. A. On a collection of nematodes from Nigerian mammals (chiefly rodents). Parasitology, 20 : 280-304. 1928.
3. CABALLERO y CABALLERO, E. Algunas filarias de mamiferos y de reptiles de las Republicas de Colombia y Panama. Ann. Inst. Biol. Mexico, 18 : 169-188. 1947.
4. CANAVAN, W. P. N. Nematode parasites of vertebrates in the Philadelphia Zoological Garden and vicinity. I. Parasitology, 21 : 63-102. 1929.
5. CANAVAN, W. P. N. Nematode parasites of vertebrates in the Philadelphia Zoological Garden and vicinity. II. Parasitology, 23 : 196-229. 1931.
6. CHANDLER, A. C. Some new genera and species of nematode worms, Filarioidea, from animals dying in the Calcutta Zoological Garden. Proc. U.S. Natl. Museum, 75 : 1-10. 1929.
7. CHANDLER, A. C. The helminths of racoons in east Texas. J. Parasitol. 28 : 255-268. 1942.
8. CHITWOOD, B. G. Note on a genus and species from *Lynx canadensis*. J. Parasitol. 20-63. 1933.
9. DESPORTES, C. *Filaria conjunctivae* Addario 1885, parasite accidental de l'homme, est un *Dirofilaria*. Ann. Parasitol. Humaine et Comparée, 17 : 380-404, 515-532. 1939-1940.
10. DESPORTES, C. *Dirofilaria repens* Railliet et Henry, 1911 possède dix papilles cèphaliques: huit submédianes et deux amphides. Ann. Parasitol. Humaine et Comparée, 18 : 192-197. 1941.
11. FAUST, E. C. Mammalian heart worms of the genus *Dirofilaria*. Festschrift Bernhard Nocht zum 80. Geburtstag. Hamburg, 131-139. 1937.
12. FAUST, E. C., THOMAS, E. P., and JONES, J. Discovery of human heartworm infection in New Orleans. J. Parasitol. 27 : 115-122. 1941.
13. FREITAS, J. F. T. de and LENT, H. Nova "Dirofilaria" parasita do aparelho circulatório de ariranha (Nematoda, Filarioidea). Rev. brasil. Biol. 9 : 377-380. 1949.
14. HALL, M. C. Nematode parasites of mammals of the orders Rodentia, Lagomorpha and Hyracoidea. Proc. U.S. Natl. Museum, 50 : 1-258. 1916.
15. KREIS, H. A. Beiträge zur Kenntnis parasitischer Nematoden. VIII. Neue parasitische Nematoden aus dem Naturhistorischen Museum Basel. Zentr. Bakt. Parasitenk. Abt. 1, Orig. 142 : 329-352. 1938.
16. LENT, H., and FREITAS, J. F. T. de. Contribução ao estudo do genero *Dirofilaria* Railliet and Henry, 1911. Mem. inst. Oswaldo Cruz, 32 : 37-54. 1937.

17. LINSTOW, O. von. Nematoden aus der Berliner Zoologischen Sammlung. Mitt. a. d. Zool. Samml. d. Mus. f. Naturk. Berlin, 1 : 3-28. 1899.

18. LINSTOW, O. von. Nematoden des Zoologischen Museum in Königsberg. Arch. Naturgeschichte, 72 : 249-258. 1906.

19. MAZZA, S. Sobre un Filarideo del corazón de coati (*Dirofilaria nasuae* n. sp.). Rev. Univ. Buenos Aires, Sect. IV, 4 : 1069-1075. 1926.

20. MENDONÇA, J. M. de. Nova espécie do gênero *Dirofilaria* Railliet and Henry, 1911 (Nematoda). Mem. inst. Oswaldo Cruz, 46 : 647-652. 1948.

21. MÖNNIG, H. O. South African parasitic nematodes. 9th and 10th Repts. Director Vet. Education and Research, South Africa, 435-478. 1924.

22. RODENWALDT, E. *Filaria kuelzii* n. sp. Arch. Schiffs-u. Tropen-Hyg. 24 : 529-535. 1910.

23. SANDGROUND, J. H. Report on the nematode parasites collected by the Kelley-Roosevelts Expedition to Indo-China, with descriptions of several new species. II. Parasites of mammals. Z. Parasitenk. 5 : 575-583. 1933.

24. SANDGROUND, J. H. On three species of filariid nematodes from sloths. Livro Jubilar Prof. Travassos, Rio de Janeiro, Brasil, 421-428. 1938.

25. SHIPLEY, A. E. On a collection of parasites from the Soudan. Arch. Parasit. 6: 604-612. 1926.

26. VAN THIEL, P. H. On some filariae parasitic in Surinam mammals, with the description of *Filariopsis asper* n.g., n.sp. Parasitology, 18 : 128-136. 1926.

27. VOGEL, H. and VOGELSANG, E. G. Neue Filarien aus dem Orang-Utan und der Ratte. Zentr. Bakt. Parasitenk. Abt. I. 117 : 480-485. 1930.

28. VUYLSTEKE, C. and RODHAIN, J. *Dirofilaria schoutedeni* n. sp. de *Colobus polykomos uelensis*. Rev. Zool. Bot. Africaines, 30 : 356-360. 1938.

29. YAMAGUTI, S. Studies on the helminth fauna of Japan. Part 35. Mammalian nematodes, 2. Japanese J. Zool. 9 : 409-439. 1941.

THE HAEMOCYTES OF THE MEDITERRANEAN FLOUR MOTH, *EPHESTIA KÜHNIELLA* ZELL. (LEPIDOPTERA: PYRALIDIDAE)[1]

By J. W. Arnold[2]

Abstract

The haemocytes of the Mediterranean flour moth were arranged in four classes, prohaemocytes, plasmatocytes, spheroidocytes, and oenocytoids, by a modification of Yeager's classification system for the haemocytes of the southern army worm. Total and differential haemocyte counts were made in each stage of the insects' development. Total cell numbers increased gradually during larval life and reached their maximum in the prepupal period. They decreased markedly during pupation and remained at a low level during adult life. Plasmatocytes and spheroidocytes accounted for more than 90% of the total cells in most stages. The plasmatocytes were in the majority during most of the larval life but were superseded by the spheroidocytes in the prepupal period. The spheroidocytes also increased in numbers when larvae were subjected to certain abnormal conditions. Histochemical tests showed that the spheroidocytes were sites for the accumulation of neutral fats just before pupation.

Introduction

The haemocytes of insects are frequently reported to play some part in defence against foreign materials, including certain insecticides. A complete understanding of this activity has not yet been obtained. Nor have the normal physiological roles of these cells been determined satisfactorily. The present investigation of the normal blood cell picture of the Mediterranean flour moth, *Ephestia kühniella* Zell., was undertaken to serve as a basis for a study of the effects of certain fumigants on the haemocytes of this species, as well as to study the normal functions of these cells.

A comprehensive study of insect haemocytes sufficiently detailed to be applied directly to the cells of different species has not yet been made. The investigation therefore involved the development of a simple, adequate cell classification, the determination of cell relationships, and the making of total and differential cell counts in each stage of development. A study of the changes in the cell picture as a result of environmental variations was also pertinent to an understanding of cell functions and to the proper interpretation of the results of subsequent toxicological studies.

Material and Methods

Insects were reared at 27° C. and 70% relative humidity (R.H.) on a substrate mixture of equal parts of whole wheat flour and corn meal. Larvae developed through six stages. Male larvae could be recognized after the third stage by the pinkish testes visible through the dorsum. Thereafter males were used exclusively. Three developmental phases were recognized in the

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sixth larval stage as follows: (*a*) a primary period of about four days during which larvae continued to feed, (*b*) a secondary period of about two days when larvae did not feed but wandered about the cages and formed cocoons, and (*c*) a prepupal period, usually of a single day.

In most cases the acetic acid vapor technique (7) was used to fix the haemocytes within the insect for smear preparations. Occasionally the hot water treatment used by Yeager (12) was employed. Blood was drawn from larval prolegs, from the wing-pads of pupae, and from a middorsal thoracic or abdominal incision in adults. A finely drawn pipette was used to remove the blood and avoid contamination of the sample with scales or dust from the insect's body.

Of the several formulations used, Giemsa blood stain (Hartman-Leddon Co., Philadelphia, Pa.) gave the most satisfactory and consistent staining. Air-dried smears were usually stained without further fixation. Occasionally they were fixed in absolute methyl alcohol for five minutes before staining. This resulted in more nearly permanent preparations but cells were somewhat less brilliantly stained and the plasma was stained pink, thereby obscuring the cells to some extent.

Total cell counts were made by standard procedures with a haemacytometer. Blood was drawn from several young larvae and adults to obtain an adequate sample. Single individuals of late larval stages provided ample amounts of blood for 1 : 100 dilution in a white cell diluting pipette. Total cell counts from first-instar larvae were impractical.

Differential cell counts were made by classifying 500 cells at random in various areas of a smear. This number was not obtainable in smears from first- and second-instar larvae and all cells in these smears were classified. Counts were made from at least 10 individuals in each stage of development and from each of the three phases recognized in the sixth larval stage.

Measurements of greatest cell and nucleus lengths and widths were made with an ocular micrometer. In most cases a minimum of 50 cells of each class were measured in each stage. This number of prohaemocytes was not obtained in all instars and oenocytoids were rarely observed.

Larvae were subjected to several abnormal conditions, including anoxia, bacterial and protozoan infection, and ligaturing. Anoxia was effected by passing a continuous stream of nitrogen through a glass tube containing the larvae. Bacteria- and protozoa-infected larvae were obtained from old or overcrowded cultures. Ligation directly behind the head was done with a fine silk thread.

The phagocytic activities of the haemocytes were observed in stained smears from larvae that had been injected with a suspension of India ink in saline as described by Yeager *et al.* (13).

Several histochemical techniques were used in studying intracellular lipoids. The techniques, which were applied to not less than five samples each of both heat-fixed and untreated blood, were as follows: (*a*) the Kay and Whitehead procedure for the detection of fats with Sudan IV (3); (*b*) the

Schultz procedure for the detection of cholesterol (3); (*c*) Baker's (1) test for the recognition of phospholipins; and (*d*) Cain's (2) procedure for the detection of neutral fats with Nile blue. The Gomori revised method for the demonstration of lipase (3) and the copper soap test (4) were used on untreated blood in an attempt to demonstrate the presence of the enzyme. Positive and negative controls were used in testing for lipase, and tests were carried out in triplicate.

Classification of the Haemocytes

Yeager's (12) classification of the haemocytes of the southern army worm, *Prodenia eridania* (Cram.), was used as a basis for the system presented here. His terminology is retained. Direct comparison of the haemocytes of *P. eridania* and *E. kühniella* revealed certain fundamental similarities and led to the simplification of the classification largely by including transitional and degenerating cell forms within their respective classes rather than considering them as separate groups. The following is a synopsis of the characteristics of the four cell classes.

Class I. Prohaemocytes

Small cells, usually round but occasionally fusiform or polymorphic; distinguished mainly by the relatively large nucleus that occupies almost the entire cell area (Fig. 1); cytoplasm, a narrow band about the nucleus and showing little if any differentiation; nucleus punctate, often largely obscured by the deeply staining cytoplasm.

Cell length, 3.3 – 13.7μ (av. 5); width 2.6 – 7.9μ.

Nucleus length, 2.6 – 7.9μ; width 2.6 – 6.6μ.

Class II. Plasmatocytes

Generally large, thin cells, round to distinctly fusiform, occasionally polymorphic; young cells often round or ovoid, showing deep cytoplasmic basophilia and a smooth periphery, resembling prohaemocytes but with a greater area of cytoplasm; mature cells (Fig. 2) distinguished by the prominent, punctate nucleus and the relatively large expanse of cytoplasm that is slightly vacuolate and moderately basophilic; cells occasionally binucleate (Fig. 8) and rarely bifurcate (Fig. 7); cytoplasmic inclusions often present in mature and degenerating cells (Fig. 10).

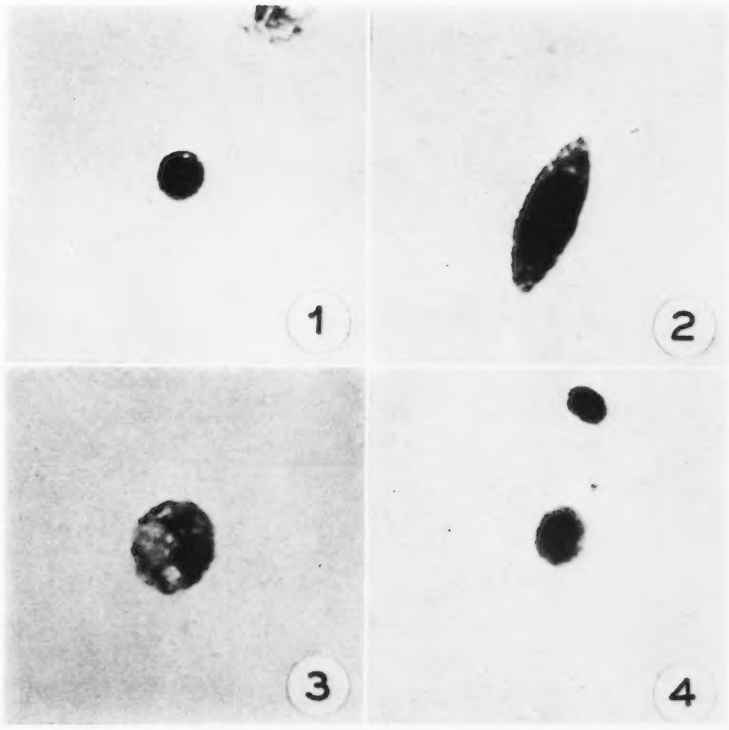
Cell length, 5.3 – 39.6μ; width 2.6 – 13.2μ.

Nucleus length, 3.3 – 11.2μ; width 2.0 – 7.9μ.

Class III. Spheroidocytes

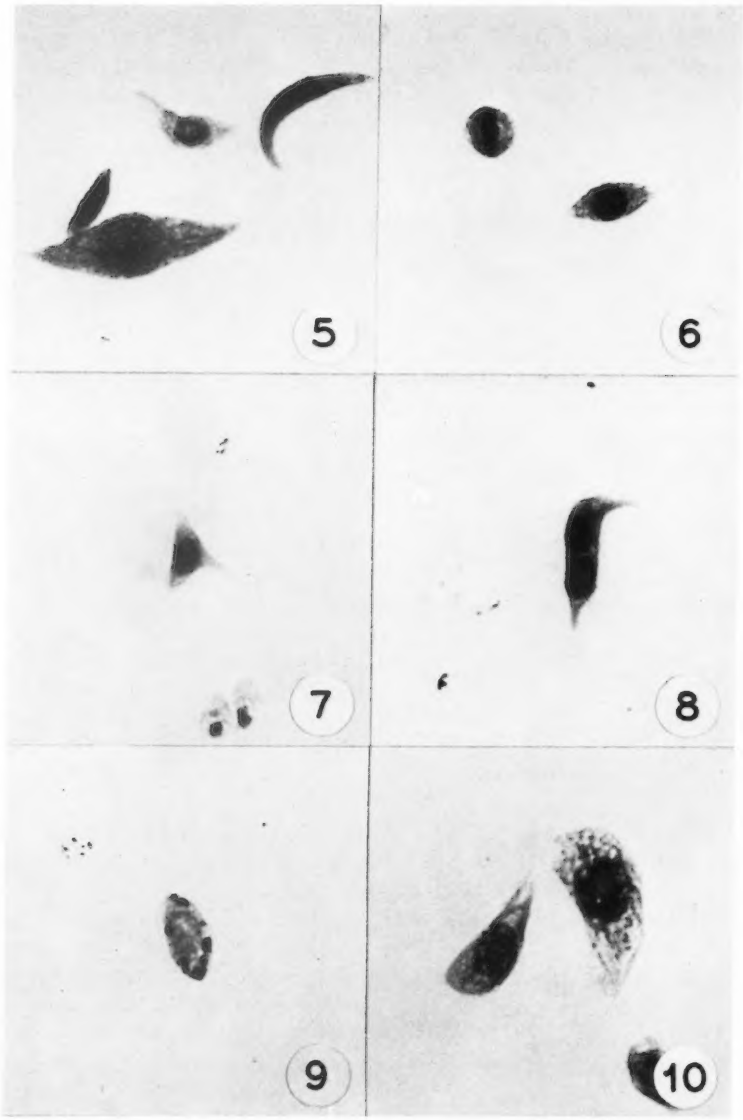
Generally small cells, almost always round or ovoid, occasionally fusiformoid; distinguished by the small, compact, eccentric nucleus and relatively large expanse of cytoplasm containing one to several distinctive vacuoles (Fig. 3) termed "spheroids" by Yeager (12); nuclei small, round or ovoid, finely punctate; rarely two or more nuclei in a cell; cytoplasm finely reticulate

PLATE I



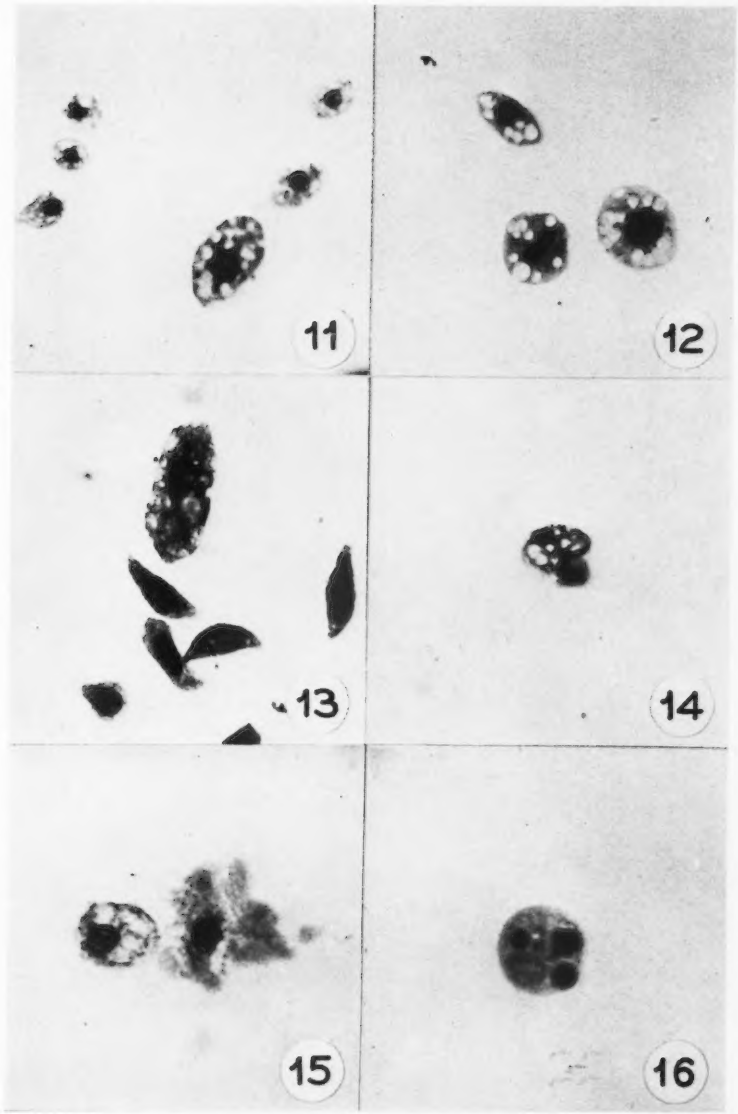
FIGS. 1–4. Photomicrographs.

FIG. 1. Typical, large prohaemocyte. (×1000) FIG. 2. Typical, mature plasmatocyte (×1500) FIG. 3. Typical, mature spheroidocyte. (×1500) FIG. 4. Typical, mature oenocytoid. (×1000)



FIGS. 5 – 10. Photomicrographs.

FIG. 5. Group of plasmatocytes showing variation in size and form. (×1500) FIG. 6. typical, mature plasmatocytes; cell on left undergoing mitotic division. (×1000) FIG. 7. Plasmatocyte showing bifurcation. (×1000) FIG. 8. Mature binucleate plasmatocyte (×1000) FIG. 9. Mature plasmatocyte showing phagocytized India ink particles in the cytoplasm. (×1000) FIG. 10. A mature and a degenerating plasmatocyte. (×1000)



FIGS. 11 – 16. Photomicrographs.

FIG. 11. Group of spheroidocytes showing variation in size and form. (×1000) FIG. 12. Group of mature spheroidocytes, one undergoing mitotic division. (×1200) FIG. 13. Large spheroidocyte showing extensive "spheroid" development; lower cells, plasmatocytes. (×1000) FIG. 14. Degenerating spheroidocyte showing "erupted" eosinophilic material. (×1000) FIG. 15. Degenerating spheroidocytes; cell on right showing complete cytoplasmic breakdown. (×1200) FIG. 16. Mature spheroidocyte from sixth-instar larva showing lipoid deposits in the cytoplasm. (×1200)

and almost always containing spheroids; spheroid contents clear to strongly eosinophilic; when eosinophilic, spheroid contents often seen spreading beyond cell border, suggesting rupture (Fig. 14); cells occasionally large and showing extensive spheroid development (Fig. 13).

Cell length, 3.3 – 17.2μ; width, 3.3 – 9.9μ.

Nucleus length, 2.6 – 6.6μ; width, 2.0 – 5.9μ.

Class IV. Oenocytoids

Generally small cells, round to ovoid, occasionally large and polymorphic; distinguished by the small nucleus and homogeneous-appearing cytoplasm (Fig. 4); nucleus small, punctate, and usually eccentric; cytoplasm homogeneous in appearance, opaque, lightly basophilic or eosinophilic, and frequently containing granules.

Cell length,* 5.9 – 15.6μ; width, 3.3 – 9.9μ.

Nucleus length, 3.3 – 5.3μ; width, 2.6 – 5.3μ.

Although based upon Yeager's work, the present classification differs considerably from the original. The cell "types" described by Yeager were not included since they represent transitional forms. Certain of his cell "classes" were deleted for the same reason: "chromophiles", "podocytes", "vermiform cells", "cystocytes", and "eruptive cells". The "chromophiles", which occurred in small numbers in *E. kühniella*, are cells intermediate between the prohaemocytes and one or more of the other cell classes. Since they are scarcely distinguishable from the prohaemocytes, they were included in that class until they showed some definite characteristic that allied them with one of the other classes. Neither "podocytes" nor "vermiform cells" appeared in as definite form in *E. kühniella* as in *P. eridania*, although somewhat similar cells were observed occasionally. Both "classes" apparently represent extremes of plasmatocyte variation, and there may be some merit in recognizing them as cell "types" in species where they occur in considerable numbers in distinctive form. Yeager's "cystocytes" occurred occasionally in *E. kühniella* and were considered a degenerate phase of plasmatocyte development. A similar suggestion was made by Jones (5). The "eruptive cells" were evidently degenerate spheroidocytes as seen from comparison of Figs. 14 and 15.

Relationships Between Haemocyte Classes

Certain relationships between the haemocyte classes became apparent from observations of transitional cell forms and subsequently from observations of cell activities and the relative numbers of cells of each class in various life stages.

The probable developmental relationships of the cell classes are illustrated in Fig. 17. The haemocytes, with the possible exception of the oenocytoids, apparently develop originally from a common source, the prohaemocytes. Development follows two diverging lines. On the one hand it results in the formation of the phagocytic cells, the plasmatocytes. On the other hand it

* *Measurements from 10 cells only.*

produces the spheroidocytes, which are potentially but not actively phagocytic. The spheroidocytes appear to develop from prohaemocytes that are smaller and more compact than normal (the "microcytes" of Yeager). Within each of these two major classes there is wide morphological variation (Figs. 5 and 11). This results from the method of cell development and from the ability of the cells to modify their form. Each class is distinctive, and cells of classes other than prohaemocytes are not modified to form those of the other.

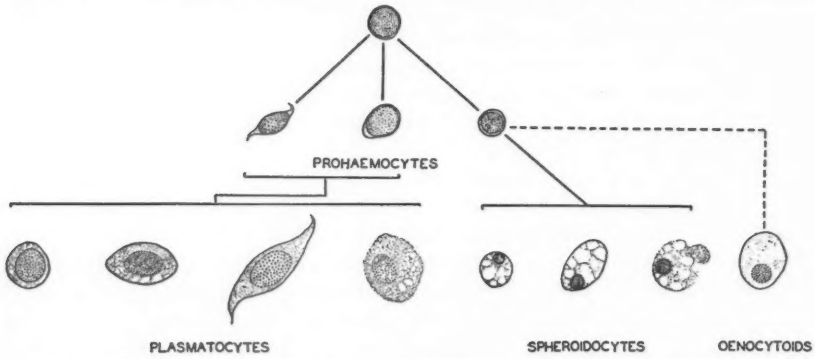


FIG. 17. Relationships between the haemocyte classes. Unbroken lines show the main trends of development; the broken line indicates a possible developmental relationship. Forms typical of stages in the development of plasmatocytes and spheroidocytes from origin to degeneration are shown (left to right).

The origin and relationships of the oenocytoids remain obscure. They occurred very rarely in the blood of *E. kühniella* and must be considered of little importance as haemocytes in this species. They do occur in greater numbers in other insects. Their general similarity to the oenocytes has led to the suggestion that they are derived from them, but Wigglesworth (10) has rejected this concept, at least for the bug *Rhodnius* sp. Their size and form suggests some relationship to the spheroidocytes. This may be appreciated by comparison of Figs. 3 and 4. It seems possible, as suggested by Yeager (12), that these cells are also derived from the prohaemocytes, possibly diverging somewhat from the line of spheroidocyte development.

Nature of the Haemocytes

The ability to modify their form was characteristic of the haemocytes, particularly of the plasmatocytes. When floating freely in the blood the plasmatocytes tended to assume a fusiform shape. This was termed the "passive condition" by Yeager (12). This was the condition of most of the cells in smears from heat- or acid-fixed insects. When attached to surfaces, or when free in the blood under unfavorable conditions, the cells tended to become round and flattened. This condition, termed "active" by Yeager, occurred in smears from unfixed larvae and could be observed when cells issuing from a wound were allowed to settle to the surface of a slide beneath a drop of immersion oil.

Despite their ability to change shape, and contrary to the reports of several investigators, the haemocytes apparently do not form pseudopodia. Extrusions resembling pseudopodia were observed in necrotic cells but these obviously did not serve for locomotion. Similar observations were made by Smith (8) on the haemocytes of the American cockroach, *Periplaneta americana* (L.).

Potentially, all classes of haemocytes appeared to be phagocytic. Generally, however, this activity was restricted to the plasmatocytes (Fig. 9), particularly during larval life. The spheroidocytes, although not especially active as phagocytes during early larval stages, frequently phagocytized India ink particles injected into sixth-instar larvae. A similar observation was made by Yeager (12) on *P. eridania.*

Although the plasmatocytes and spheroidocytes originate from a common source, and this process continues to some extent throughout development, major cell increases apparently result largely from mitotic division of cells of each class. Increase in the relative numbers of a particular cell class coincided with increased mitotic activity of that class (Tables II and III). Mitotically dividing cells were observed among the prohaemocytes, plasmatocytes (Fig. 6), and spheroidocytes (Fig. 12). They were not observed among the oenocytoids.

The haemocytes increased somewhat in size during larval development, cells of maximum size occurring mainly in the sixth stage. Cells in all stages of development, and therefore in a great variety of sizes, occurred in each stage, but greater numbers of young, small cells were present in early larval life. The cells, particularly the spheroidocytes, were smaller in newly emerged adults than in late larval stages, suggesting that they are new cells developed during pupation.

Total Haemocyte Counts

The variations in the average number of haemocytes per cubic millimeter of undiluted haemolymph at various stages in the life cycle are illustrated in Fig. 18. The actual results were as follows: 2nd instar, 46,166 cells per cu. mm.; 3rd, 58,583; 4th, 56,292; 5th, 69,250; 6th (primary period), 65,320; prepupa, 73,500; pupa (1st day), 18,000; pupa (2nd day), 12,250; pupa (3rd day), 7250; pupa (4th day), 4000; adult (1st day), 6000. The number of counts made in each stage was as follows: 2nd, 6; 3rd, 4th, and 5th, 12 each; 6th and prepupa, 25 each; pupa, 5 on each of the first three days; adult, 5 on the day of emergence.

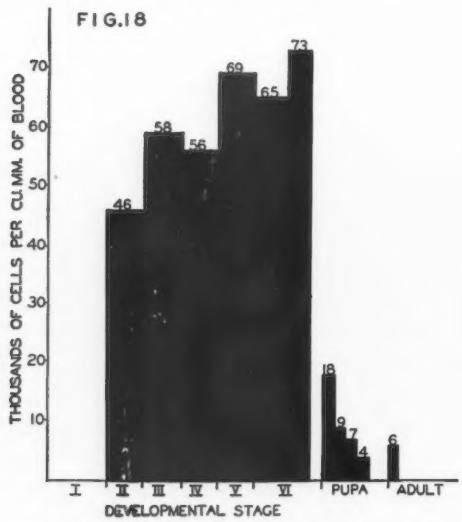
The cells increased in numbers at a relatively constant rate during larval life and reached a maximum in the prepupal period. They declined very rapidly at pupation and reached minimum numbers during that stage. A slight increase occurred at emergence of the adults but cell numbers in this stage were markedly lower than during larval development. Apparently the

haemocytes of this species are most active during larval life and are not required during pupation for the removal of histolyzed tissues. Their small numbers in adults implies a lesser degree of activity than occurred in larvae.

Differential Haemocyte Counts

The results of differential haemocyte counts are given in Table I. The variations in relative numbers of the three major cell classes during the life cycle are illustrated in Fig. 19.

The prohaemocytes comprised a relatively small fraction of the haemocyte population in all except the first larval stage. The plasmatocytes occurred in comparatively small numbers in first-instar larvae, increased in the second stage presumably at the expense of the prohaemocytes, and fluctuated about the 70% level until the end of the fourth stage. Thereafter their numbers gradually declined. Their relative increase during pupation resulted from the rapid and almost complete degeneration of the spheroidocytes at that time. The spheroidocytes formed approximately the numerical reciprocal of the plasmatocytes. They occurred in comparatively small numbers until the end of the fourth stage and then increased gradually to a maximum in the prepupal period. They degenerated very rapidly after the onset of pupation but their relative numbers were again high at the emergence of the adults. They gradually declined on successive days of adult life. The oenocytoids occurred very rarely. They were not observed during the first two larval stages or in the prepupal period.



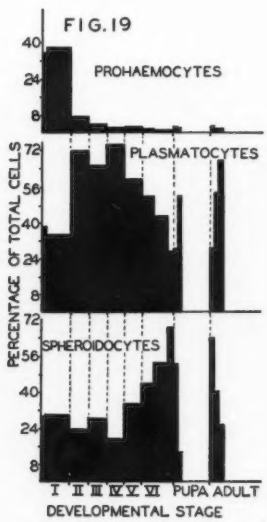


FIG. 18 (left). Variations in total cell numbers during the life cycle (width of each column proportional to the number of days in the stage).

FIG. 19 (right). Variations in percentages of cells of each of the three main haemocyte classes during the life cycle (width of each column proportional to the number of days in the stage).

TABLE I

RELATIVE CELL NUMBERS IN HAEMOCYTE CLASSES DURING THE LIFE CYCLE

Stage	Percentage of cells							
	Prohaemocytes	Plasmatocytes	Spheroidocytes	Oenocytoids	Degenerate cells	Mitotic figures	Fusiform cells	Number counted
First instar[1]	33.6	37.6	28.8	0	0	0.10	7.7	465
First instar[2]	36.5	34.3	29.2	0	0	0.10	8.9	1000
Second instar	6.4	71.0	22.6	0	0	0.20	27.7	1090
Third instar	2.9	67.1	28.4	(9 cells)	1.6	0.22	30.4	12,607
Fourth instar	1.6	78.4	19.0	(7 cells)	1.0	0.15	37.7	14,500
Fifth instar	1.8	61.3	35.4	(9 cells)	1.5	0.20	30.8	15,000
Sixth instar[3]	1.3	53.1	43.8	(7 cells)	1.8	0.26	26.2	15,000
Sixth instar[4]	0.6	44.5	53.7	(8 cells)	1.2	0.20	27.4	15,000
Prepupa	0.5	28.4	68.7	0	2.4	0.09	16.4	15,000
Pupa, 1st day	1.6	29.3	53.2	(1 cell)	15.9	0.01	20.7	11,500
Pupa, 2nd day	1.4	53.8	13.7	(12 cells)	31.1	0.22	38.1	11,500
Imago, 1st day	3.2	30.5	63.9	(1 cell)	2.4	0.14	17.8	10,000
Imago, 2nd day	0.9	54.2	41.6	(2 cells)	3.3	0.10	—	2000
Imago, 3rd day	1.0	68.6	26.9	0	3.5	0.05	—	2000

[1] *Just hatched.*
[2] *At least two days old.*
[3] *Primary period.*
[4] *Secondary period.*

Small numbers of degenerate plasmatocytes and spheroidocytes appeared in the third larval stage, indicating a cell-life of approximately 10 days. Degenerating cells were relatively constant in numbers during the remainder of larval life. They increased markedly at pupation. This increase coincides with the observed decrease in total cell numbers and supports the suggestion that the haemocytes are not active in the removal of broken tissues during pupation in this species. Few degenerate cells occurred in newly emerged adults, but their numbers increased slightly on successive days.

Generally one or two cells per 1000 were dividing mitotically at a given time. This was relatively constant throughout development. Slight increases were observed in the sixth and pupal stages.

Fusiform cells, whose numbers indicated to some extent the degree of cell activity, fluctuated about the 30% level until the prepupal period, when cell activity increased. It decreased during pupation and was high in newly emerged adults.

Effects of Abnormal Conditions on the Haemocytes

Moderate variations in temperature did not noticeably affect the haemocytes. Exposure of sixth-instar larvae to 15° C. for 48 hr. caused no apparent change in the cell picture. Similarly, the cells appeared normal in sixth-instar larvae that were reared from the fourth stage at 17° C. Exposure of sixth-instar larvae to 34° C. for 48 hr. caused an apparent small increase in the numbers of degenerate cells, but no other noticeable changes.

Anoxia caused no apparent change in the haemocytes. Three groups of five larvae that were sacrificed immediately after nitrogen treatments of one, two, and three hours, respectively, showed normal cell pictures. No counts were made.

Occasionally smears contained large numbers of bacilli. In such smears the spheroidocytes occurred in unusually large numbers, often more than 50% of the total cells. Protozoa-infected larvae were also occasionally examined. These parasites were identified as *Microsporidia* sp. (9). In young, infected larvae total cell numbers were not significantly different from normal (average count of 63,000 cells per cu. mm. from five infected third-instar larvae). In later stages the haemolymph was occupied almost entirely by parasite spores and the haemocytes were almost completely destroyed.

Starvation caused a rapid change in the relative numbers of spheroidocytes. The results of differential cell counts from five fourth-instar larvae that were starved for 24 hr. are given in Table II. The significant increase in relative numbers of spheroidocytes and their mitotic index, and the increase in numbers of degenerate cells are evident from comparison with the same stage in Table I. Generally, the cell picture was similar to that of larvae in the prepupal period.

To determine whether starvation might have induced the early onset of pupation and resulted in the observed changes, metamorphosis was blocked and a condition of starvation was created by ligaturing fourth-instar larvae

behind the head (11). The results of differential cell counts from five ligatured larvae after 24 hr. are given in Table III. The results are almost identical with those of starved larvae. Evidently starvation, and not metamorphosis, was responsible for the change in the cell picture.

TABLE II

THE HAEMOCYTE PICTURE DURING STARVATION OF 4TH-INSTAR LARVAE

Individual	Number of cells								
	Prohaemocytes	Plasmatocytes		Spheroidocytes		Oenocytoids	Mitotic Cells		Fusiform cells
		Normal	Abnormal	Normal	Abnormal		II*	III**	
1	0	62	42	350	46	0	1	5	15
2	2	174	68	203	53	0	1	1	55
3	1	111	56	303	28	1	0	1	25
4	0	100	138	219	41	2	0	4	6
5	0	120	95	205	80	0	2	1	20
Mean (approx.)	1	113	80	256	49	1	1	2	24
Percentage	0.1	22.7	16.0	51.2	9.9	0.1	0.2	0.5	4.8

* *Plasmatocytes.*
** *Spheroidocytes.*

TABLE III

THE HAEMOCYTE PICTURE FOLLOWING LIGATION OF 4TH-INSTAR LARVAE BEHIND THE HEAD

Individual	Number of cells								
	Prohaemocytes	Plasmatocytes		Spheroidocytes		Oenocytoids	Mitotic Cells		Fusiform cells
		Normal	Abnormal	Normal	Abnormal		II*	III**	
1	1	123	69	277	30	0	0	4	19
2	1	92	85	242	80	0	0	1	35
3	1	92	61	285	58	3	1	3	15
4	2	113	113	205	65	2	2	2	22
5	0	261	5	225	9	0	0	1	120
Mean (approx.)	1	136	67	247	48	1	1	2	42
Percentage	0.2	27.2	13.3	49.4	9.7	0.2	0.1	0.4	8.4

* *Plasmatocytes.*
** *Spheroidocytes.*

Relationship of the Spheroidocytes to Fat Metabolism

In the secondary and prepupal period of the sixth larval stage, the spheroidocytes contained large amounts of a lipoid material. The lipoid appeared rarely in cells in early larval stages, but it occurred in gradually increasing numbers of cells as pupation approached. It occurred in almost every spheroidocyte in the prepupal period. At the same time it appeared in much smaller amounts in some of the plasmatocytes. Munson and Yeager (6) reported similar findings in *P. eridania* but did not mention the lipoid was restricted to the spheroidocytes.

The lipoid occurred as small globules in the cytoplasm of the spheroidocytes and also as larger masses that appeared to fill or surround the spheroids (Fig. 16). Only small globules appeared in the plasmatocytes. Histochemical tests showed that it was composed largely of neutral fats with possible traces of phospholipins. Tests for cholesterol were negative. Similar results were obtained when these tests were applied to frozen sections of fat body. The enzyme lipase was not detected in the cells either in young larvae, where the lipoid did not occur, or in prepupae.

Fatty degeneration was apparently not involved in the appearance of the lipoid, since it appeared in normal young cells and mitotically dividing cells as well as in old cells. It was apparently not the result of phagocytosis of lipomicrons, since little appeared in the phagocytic plasmatocytes. Presumably it either resulted from the conversion of bound fats to a free condition or was synthesized in the cells from carbohydrates and/or proteins. Munson and Yeager (6) found that in *P. eridania* fat appeared and continued to be found in the haemocytes as glycogen disappeared from them. They also noted that haemocytes of larval stages contained fat inclusions if the larvae ingested sufficient fat. Presumably the spheroidocytes are active in fat metabolism. This is supported by the fact that they increase in numbers under certain conditions, as starvation and bacterial infection, when reserve storage is being utilized.

General Discussion

The arrangement of the haemocytes into four major groups, each having distinctive cytological features and comparatively restricted functions, should be of value to other workers. It provides a simple basis for the classification of the cells of other holometabolous species. With slight modification, particularly in the description of specific class characteristics, the writer has found it applicable to the haemocytes of a number of widely separate species, including the yellow meal worm, *Tenebrio molitor* L.; the cadelle, *Tenebroides mauritanicus* (L.); the southern army worm, *Prodenia eridania* (Cram.); the wax moth, *Galleria mellonella* (L.); and the housefly, *Musca domestica* L.

The relationships observed between the cell classes conform in general to Yeager's (12) suggestions but are less complex because of the simplification of the classification. The prohaemocytes are the stem cells from which the plasmatocytes, spheroidocytes, and possibly the oenocytoids arise. Develop-

ment from the prohaemocytes accounts for part of the numerical increase in cells during all stages. Major increases result from mitotic division of cells of each class. The plasmatocytes and the spheroidocytes are the two functionally active classes and are related only in origin. The oenocytoids, whose origin is still somewhat obscure, appeared very rarely in this species. They appear to be a distinct class and are more numerous in other species. Each class is composed of cells of varied size and form as a result of differences in age and activity. This results from the manner of development, new cells continually being formed from the prohaemocytes and from the division of existing cells. There is no evidence of haematopoietic tissues.

It has been generally reported that insect haemocytes increase in numbers at pupation and assist in the removal of autolyzed tissues. This view has been recently supported by Jones (5). He felt that the numerical reduction of free-floating cells resulted from their confinement to the internal interstices of metamorphosing tissues. This evidently is not the case in *E. kühniella*. In this species the total cell numbers decrease during pupation as the number of degenerating cells increase, and the total cell numbers are much lower in emerging adults than in the larvae. These results support Yeager's suggestion that larval haemocytes are largely destroyed during pupation and are replaced from the imaginal disks at that time.

The results of differential cell counts are generally similar to those obtained by Yeager with *P. eridania*. Early larval life is a period of haemocyte differentiation, as shown by the large numbers of prohaemocytes present. The rapid decline in their numbers coinciding with the increase in plasmatocytes in the second larval stage strongly suggests that their differentiation is directed largely toward the formation of plasmatocytes at that time. The phagocytic activities of the plasmatocyctes are apparently required during larval development and they then form the numerically largest group. The increase in spheroidocytes at the end of larval life and their rapid decline after the onset of pupation suggests some particular function associated with the pupation process. Two changes are noticeable in these cells at pupation. They become more phagocytic, and they become loaded with neutral fats. Fat storage and phagocytosis are apparently unrelated since fat deposition occurs to a negligible extent in the phagocytic plasmatocytes. Evidently the presence of fats in the spheroidocytes at this time is significant. It implies the ability of these cells to synthesize fats from carbohydrates and/or proteins, thereby contributing to the energy metabolism of the pupa. This suggestion is supported by the fact that the spheroidocytes increase in numbers under conditions such as bacterial infection and starvation when presumably energy reserves are being depleted.

Conclusions

The haemocytes of *E. kühniella* may be arranged in four classes on the basis of distinctive cytological features. Two of these classes, the plasmatocytes and the spheroidocytes, are functionally important in this species. The former are phagocytes and apparently the latter are primarily concerned in

fat metabolism. Of the other two classes, the prohaemocytes are the stem cells from which the other classes originate, and the oenocytoids, which appear rarely, are of unknown function. Cell increases occur partly through growth and differentiation of the prohaemocytes, and partly by mitotic division of other cells. The rate of numerical cell increase exceeds degeneration during larval life and results in a gradual increase in total cell numbers until pupation. Early larval stages are characterized by a majority of plasmatocytes. Spheroidocytes form the numerically largest group near the end of larval life. They are loaded with fat globules at pupation. Cell numbers decline rapidly at pupation and are comparatively small in adults. Under certain conditions of stress the spheroidocytes increase in numbers. It is suggested that their increase is significant and implies an ability to synthesize fats when conditions require excessive utilization of food reserves.

References

1. BAKER, J. R. The histochemical recognition of lipine. Quart. J. Microscop. Sci. 87 : 441–470. 1946.
2. CAIN, A. J. The use of Nile blue in the examination of lipoids. Quart. J. Microscop. Sci. 88 : 383–392. 1947.
3. GLICK, D. Techniques of histo- and cytochemistry. Interscience Publishers, Inc., New York. 1949.
4. HAWK, P. B., OSER, B. L., and SUMMERSON, W. H. Practical physiological chemistry. The Blakiston Company, Toronto. 1947.
5. JONES, J. C. The normal haemocyte picture of the yellow mealworm (*Tenebrio molitor*). Iowa State Coll. J. Sci. 24 : 355–361. 1950.
6. MUNSON, S. C. and YEAGER, J. F. Fat inclusions in the blood cells of the southern armyworm (*Prodenia eridania*). Ann. Entomol. Soc. Am. 37 : 396–400. 1944.
7. SHULL, W. E. and RICE, P. L. A method for the temporary inhibition of coagulation in the blood of insects. J. Econ. Entomol. 26 : 1083–1089. 1933.
8. SMITH, H. W. The blood of the cockroach. New Hampshire Agr. Expt. Sta. Tech. Bull. 71. 1938.
9. STEINHAUS, E. A. Insect microbiology. Comstock Pub. Co., Inc., Ithaca, New York. 1947.
10. WIGGLESWORTH, V. B. The principles of insect physiology. Methuen & Co. Ltd., London. 1947.
11. WILLIAMS, C. M. The metamorphosis of insects. Sci. American, 182 : 24–28. 1950.
12. YEAGER, J. F. The blood picture of the southern armyworm (*Prodenia eridania*). J. Agr. Research, 71 : 1–40. 1945.
13. YEAGER, J. F., MCGOVRAN, E. R., MUNSON, S. C., and MAYER, E. L. Effect of blocking haemocytes with Chinese ink and staining nephrocytes with trypan blue upon the resistance of the cockroach (*Periplaneta americana*) to sodium arsenite and nicotine. Ann. Entomol. Soc. Am. 35 : 23–40. 1942.

EFFECTS OF CERTAIN FUMIGANTS ON HAEMOCYTES OF THE MEDITERRANEAN FLOUR MOTH, *EPHESTIA KÜHNIELLA* ZELL. (LEPIDOPTERA: PYRALIDIDAE)[1]

By J. W. Arnold[2]

Abstract

A study was made of the effects of three fumigants, dichloroethyl ether, carbon tetrachloride, and methyl bromide, on the haemocytes of the Mediterranean flour moth. Cytological changes, total cell numbers, and the relative numbers of cells of each class were noted at intervals during fumigation and in recovering and moribund larvae after treatment. With each compound, fumigation caused passive–active cell changes and pathological conditions leading to cell degeneration and also resulted in significant decreases in cell numbers. Total cell numbers decreased further in individuals that failed to recover from fumigation. Recovery was associated with regenerative changes that resulted in increased cell numbers. The spheroidocytes were the principal cells involved in the increases and they were loaded with globules of neutral fats at that time. It is suggested that the spheroidocytes are associated with recovery, possibly through an ability to aid in the creation of fat reserves for energy metabolism under unfavorable conditions.

Introduction

The possibility that the haemocytes of insects may play some role in insect resistance to insecticides is the basis for this investigation. This possibility has been frequently suggested and was recently supported by the works of Yeager and Munson (9) and of Yeager *et al.* (8). The effects of numerous fumigants on blood coagulation and the gross effects on haemocytes of the oriental cockroach, *Blatta orientalis* L., have been studied (6) but no detailed investigation of the effects of fumigants on individual cell classes has previously been made.

In the experiments reported below, the effects of certain fumigants on the haemocytes were studied to determine whether any particular class of cells might be active in defence. Presumably, if a specific class of haemocytes were concerned it would be evident from changes in cell morphology and/or from an increase in the numbers of cells of the active class. The present work involved the determination of changes in total cell numbers, cytological changes, and variations in the relative numbers of cells of each class with each fumigant. Observations were made at intervals during fumigation with median lethal concentrations of the gases and in moribund and recovering individuals after treatment. Interpretation of results was possible by reference to the normal haemocyte picture of this species (1).

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Contribution No. 2994, Division of Entomology, Science Service, Department of Agriculture, Ottawa, Canada. Part of a thesis submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy at the University of Western Ontario, London, Ont.

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Material and Methods

Test insects were in the primary period* of the sixth larval stage. Male larvae were used exclusively. All were reared as described previously (1). In certain instances, fourth-instar larvae were used to verify results.

The three fumigants used (all from Eastman Kodak Co., Rochester, N.Y.) were as follows: (*a*) β, β'-dichloroethyl ether, (*b*) carbon tetrachloride, (*c*) methyl bromide.

Median lethal concentrations of each fumigant were determined by standard procedures (7). These dosages were used in the experiments so that approximately equal numbers of recovering and dying insects were obtained for study. Separation of larvae into the two groups, moribund and recovering, was made after a 48-hr. recovery period. The MLD_{50} for dichloroethyl ether was 2.3 mgm. per liter; for carbon tetrachloride, 194; and for methyl bromide, 0.5.

Total cell counts were made from 25 individuals of each of the following three groups: (*a*) larvae at the end of the five-hour fumigation, (*b*) moribund larvae 48 hr. after fumigation, and (*c*) recovering larvae 48 hr. after fumigation. These counts were made after treatment with each of the three fumigants.

Differential cell counts, involving classification of 500 cells at random from a smear, were made from 20 larvae at the following periods with each fumigant, except methyl bromide: (*a*) five minutes after introduction of the fumigant, (*b*) two and one-half hours after the beginning of fumigation, (*c*) end of the five-hour fumigation, (*d*) 48 hr. after fumigation (20 moribund, 20 recovering). No counts were made at the mid-point of fumigation with methyl bromide. Cytological observations were made during the differential counting. Cell activity as indicated by the number of fusiform cells and the mitotic index was also noted.

With dichloroethyl ether, the effect of a series of fumigations of low concentration was also studied. A number of larvae were exposed to low concentrations of the gas (approx. 0.1 mgm. per liter) at 48-hr. intervals from the second to the sixth stage. Total and differential cell counts were made from the survivors in the primary period of the sixth stage.

Histochemical tests for phospholipins, cholesterol, and neutral fats as previously described (1) were carried out on lipoids that appeared in spheroidocytes during recovery from fumigation. The total fat content of the blood was determined by ether extraction of blood that was deposited on fat-free filter paper. Three tests were made, each with blood from 50 larvae. A micro-Soxhlet extractor was used. Ether extractions were also carried out on whole larvae that had been dried and pulverized. Extractions were from three groups of 50 normal larvae and from the same number of larvae recovering from carbon tetrachloride fumigation.

* *First four days, during which larvae continued to feed.*

Results

In general the fumigants caused similar reactions. Unless otherwise stated the results apply to each fumigant.

Total Cell Counts

The results of total cell counts are illustrated in Fig. 1. Cell numbers decreased during fumigation. This decrease was highly significant except for carbon tetrachloride, where regenerative changes masked it. It should be pointed out that few larvae were actually dead at the end of fumigation and the samples used therefore included insects that would have subsequently died as well as some that would have recovered. After the 48-hr. recovery period the cell numbers increased significantly in recovering individuals but continued to decrease in moribund larvae. Regenerative changes were particularly rapid during carbon tetrachloride fumigation. Cell numbers were greater than normal 48 hr. after fumigation in recovering individuals but normal numbers had not been reached in that period with the other two fumigants.

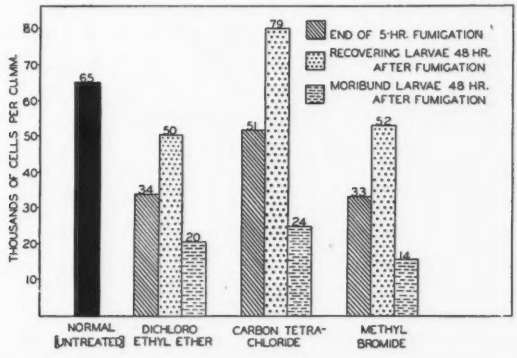


FIG. 1. Changes in total haemocyte numbers after fumigation.

Haematological Changes

In general, cell changes included passive to active changes (9) followed by some cell agglutination, distortion, and disintegration. Except for agglutination, the changes were similar to normal degenerative phases but they proceeded at a much more rapid rate.

The cell classes differed somewhat in their reactions to the fumigants. The prohaemocytes were not noticeably affected at the concentrations used. Similarly, the few oenocytoids that were noted appeared to be unaffected. The plasmatocytes were the first cells to show changes. The majority lost their fusiform shape within the first five minutes of fumigation (Figs. 2 and 14) and many became distinctly flattened. Subsequently they showed a tendency to swell (Figs. 9, 10, 12, and 15) and became somewhat achromophilic, the cytoplasm became excessively vacuolated (Figs. 9 and 10), and

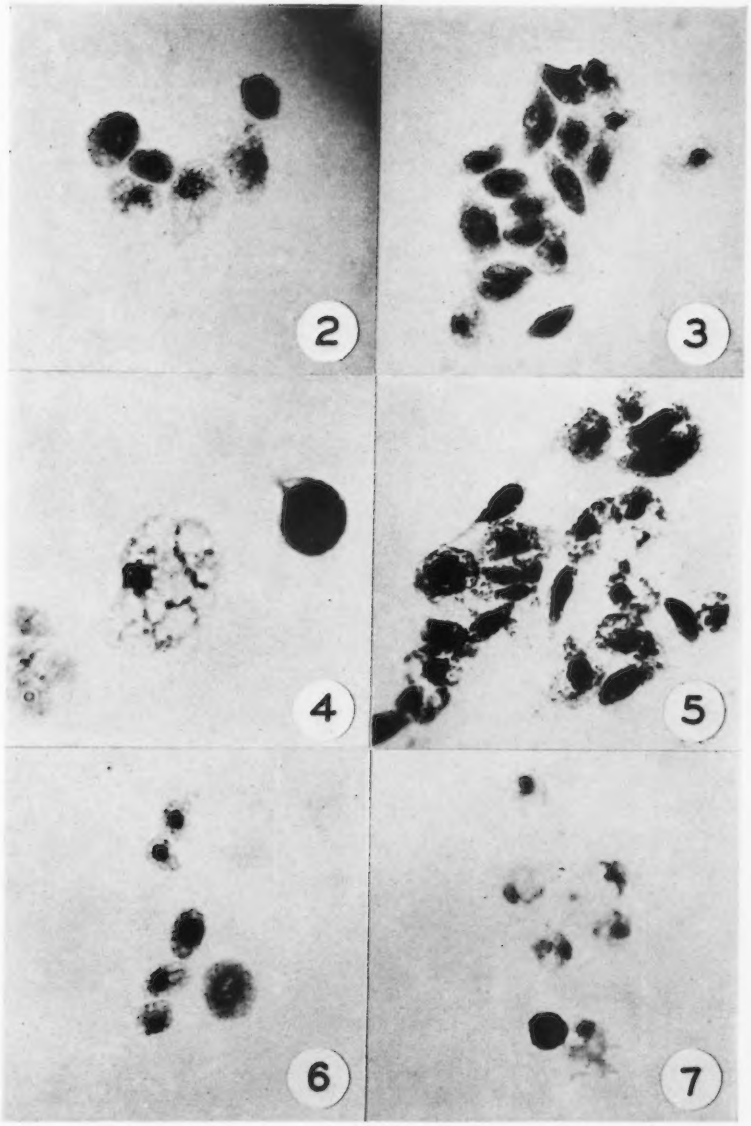
the periphery became ragged (Figs. 5, 10, 16). Nuclear distortion, fragmentation, and extrusion followed the preliminary stages of cytoplasmic breakdown (Figs. 3, 5, 10, 12, and 19). The spheroidocytes were somewhat less rapidly affected than the plasmatocytes. Some became eosinophilic and their vacuolar contents extruded into the plasma. Generally they became less turgid and occasionally assumed bizarre shapes (Fig. 4). Rupture of the cell membrane and general dissolution of the cytoplasm followed (Figs. 7, 9, 10, 12, 17, and 19). The nuclei usually retained their compact form for some time after being completely dissociated from the cytoplasm. Clumping of cells of all kinds occurred, at first occasionally and later to a large extent. Usually the clumps were composed of massed cells of all kinds (Figs. 3 and 5). Often, however, agglutination of similar cells occurred. This was particularly true of the spheroidocytes, which formed ringlike groupings of various numbers of cells contacting each other at a single point (Fig. 8).

Differential Cell Counts

The results of differential cell counts are given in Table I. The relative numbers of prohaemocytes and oenocytoids did not change appreciably during or after fumigation with any of the compounds. However, the plasmatocyte–spheroidocyte relationship was markedly altered (Fig. 20). With dichloroethyl ether, approximately equal numbers of plasmatocytes and spheroidocytes were affected at first. This was followed by a period of rapid plasmatocyte regeneration. During the recovery period the picture was reversed, the spheroidocytes then outnumbering the plasmatocytes (Fig. 6). This occurred in moribund as well as in recovering insects. However, in the moribund larvae the majority of the cells had degenerated at 48 hr. (Fig. 7). With the other two fumigants there was no marked increase in plasmatocytes during fumigation. Both classes were equally affected at first, but regeneration was thereafter greater among the spheroidocytes. They outnumbered the plasmatocytes at the end of fumigation and this relationship was exaggerated in recovering insects after 48 hr. (Figs. 11 and 18). In moribund insects a similar condition had apparently developed but the cells had subsequently degenerated (Fig. 19).

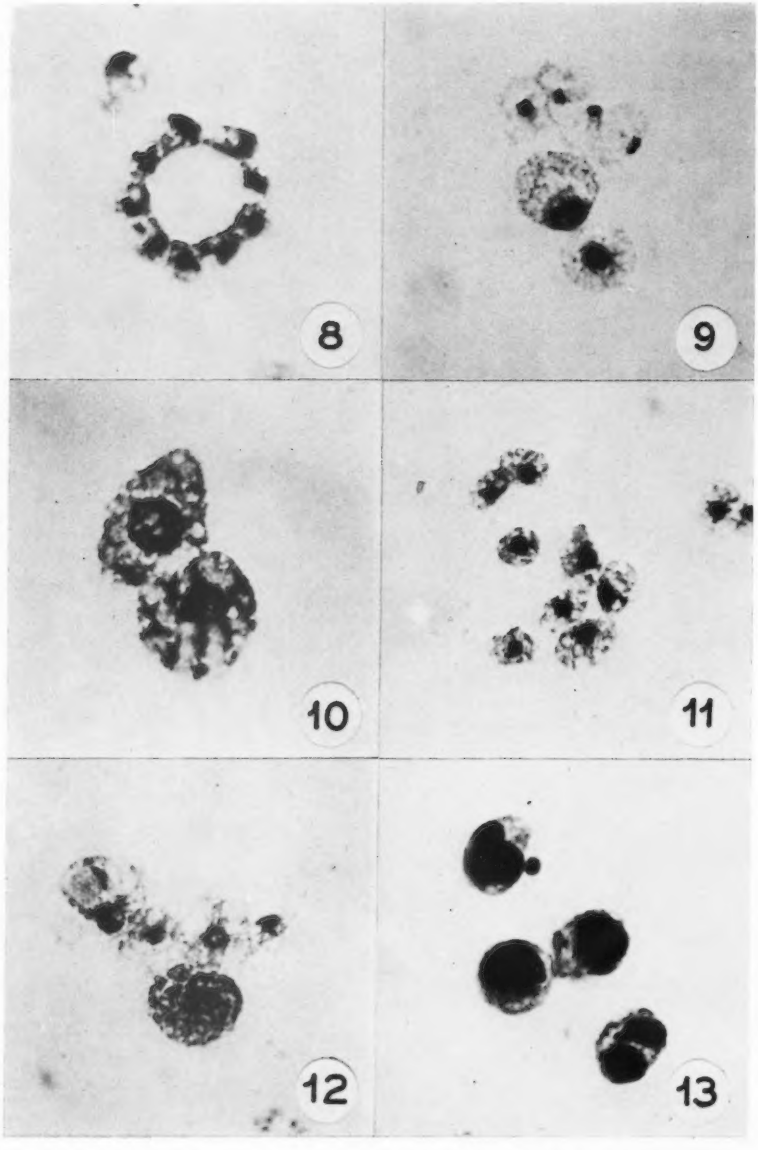
The extent of cell degeneration differed with each fumigant. Dichloroethyl ether caused the destruction of only 20–30% of the cells during the five-hour fumigation, and similar numbers of degenerate cells occurred in recovering larvae. With carbon tetrachloride, over 50% of the cells were degenerate at the mid-point of fumigation but regeneration reduced this percentage thereafter. About 30% of the cells were destroyed by methyl bromide but few degenerate cells were present in recovering individuals. With each fumigant about 80% of the cells were degenerate in moribund insects 48 hr. after treatment.

Passive-active cell changes were evident within the first five minutes of fumigation with each compound. During that time the number of fusiform cells decreased significantly and remained comparatively constant throughout fumigation.



Photomicrographs of haemocytes during and after fumigation with dichloroethyl ether.

FIG. 2. Group of plasmatocytes and spheroidocytes after five-minute exposure to fumigant, showing passive–active change and some cytolysis. (×1000) FIG. 3. Group of plasmatocytes and spheroidocytes after two- and one-half-hour exposure to fumigant, showing agglutination and nuclear and cytoplasmic degeneration. (×1000) FIG. 4. Spheroidocyte and young plasmatocyte after five-hour exposure to fumigant. Spheroidocyte shows advanced stage of cytolysis. (×1500) FIG. 5. Group of haemocytes after five-hour exposure to fumigant, showing agglutination and necrosis. (×1000) FIG. 6. Haemocytes from a recovering larva 48 hr. after fumigation. (×1000) FIG. 7. Haemocytes from a moribund larva 48 hr. after fumigation, showing complete breakdown. (×1000)



Photomicrographs of haemocytes during and after fumigation with carbon tetrachloride.

FIG. 8. Ringlike grouping of spheroidocytes after two-and one-half-hour exposure to the fumigant. (×1000) Fig. 9. Group of haemocytes after five-hour exposure to fumigant, showing extensive degeneration. (×1000) FIG. 10. Plasmatocyte (upper cell) and spheroidocyte after five-hour exposure to fumigant. Plasmatocyte shows nuclear and cytoplasmic breakdown. Spheroidocyte shows some evidence of cytoplasmic degeneration. (×1500) FIG. 11. Spheroidocytes from a recovering larva 48 hr. after fumigation. (×1000) FIG. 12. Haemocytes from a moribund larva 48 hr. after fumigation, showing complete breakdown. (×1000) FIG. 13. Spheroidocytes from a recovering larva showing intracellular lipoids (black). (×1200)

The mitotic index* varied somewhat under the influence of the different fumigants, coinciding generally with the variations in cell numbers. During dichloroethyl ether fumigation the index increased, largely on the part of the plasmatocytes. During recovery it was normal, but largely in favor of the spheroidocytes. With carbon tetrachloride no change occurred in the index until the end of fumigation. At that time it was approximately twice normal, largely as a result of spheroidocyte activity. It increased further in recovering individuals. With methyl bromide a sudden, unaccountable increase in the index occurred after five minutes of fumigation. The index was normal in recovering larvae but the majority of dividing cells were spheroidocytes. With each fumigant very few mitotic cells were observed in moribund larvae after the 48-hr. recovery period.

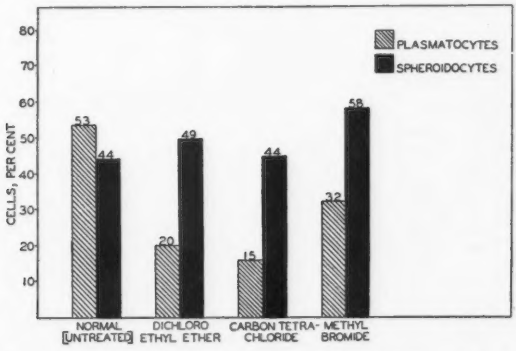


FIG. 20. Relative numbers of plasmatocytes and spheriodocytes in larvae recovering from fumigation.

Haemocyte Picture After a Succession of Light Fumigations

The average total cell count from 25 sixth-instar larvae that had been subjected to low concentrations of dichloroethyl ether at 48-hr. intervals from the second stage was 76,180. This number is significantly greater than that for normal individuals (65,320).

The results of differential counts are included in Table I. The relative cell numbers in haemocyte classes were generally similar to those recorded in larvae recovering from fumigation at MLD_{50}. The most significant feature was the plasmatocyte–spheroidocyte relationship, the spheroidocytes outnumbering the plasmatocytes. The number of dividing spheroidocytes was about twice normal. Passive cells were few in number.

Lipoids in the Haemocytes of Recovering Larvae

Recovery from the effects of each fumigant was associated with an increase in the numbers of spheroidocytes. These cells in recovering larvae were loaded with lipoid globules (Fig. 13), and were similar in appearance to the

* *Number of mitotically dividing cells/total cells.*

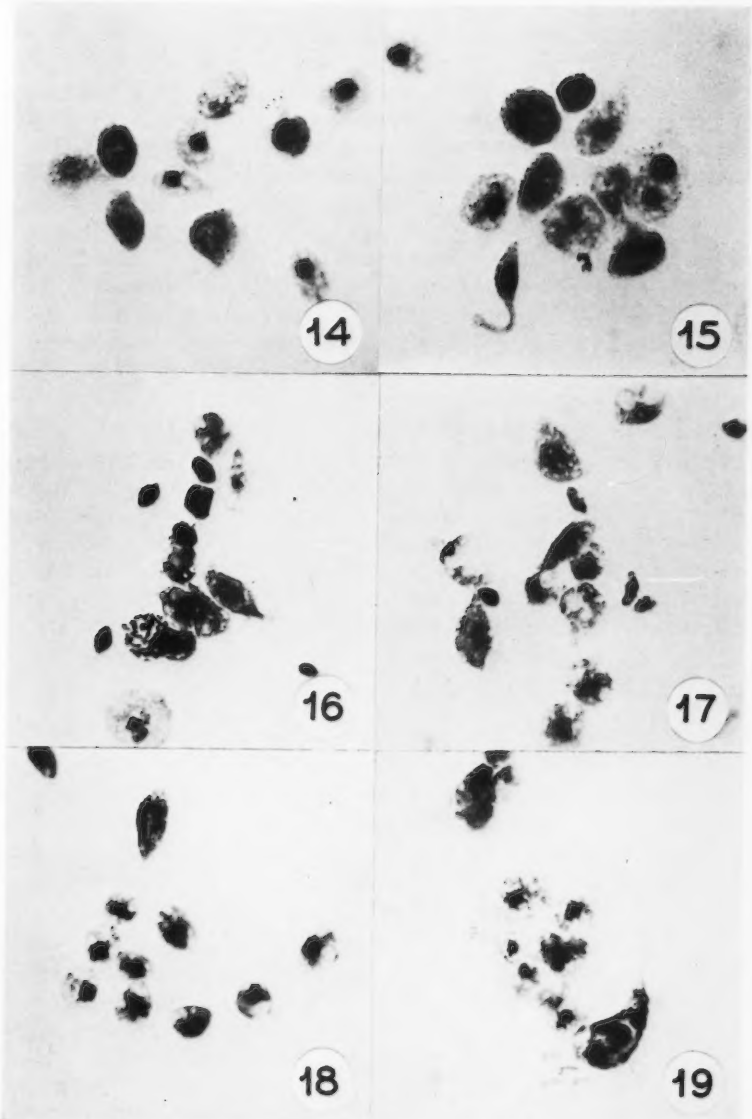
TABLE I

PERCENTAGE OF CELLS IN HAEMOCYTE CLASSES AFTER TREATMENT WITH THREE FUMIGANTS

Treatment	Percentage of cells								
	Prohaemocytes	Plasmatocytes	Spheroidocytes	Oenocytes	Degenerate cells	Mitotic figures: Prohaemocytes	Mitotic figures: Plasmatocytes	Mitotic figures: Spheroidocytes	Fusiform cells
Dichloroethyl ether									
5 min.	0.3	40.0	23.5	(2 cells)	36.2	0	0.19	0.18	10.3
2½ hr	0.5	40.9	38.8	0	19.8	0	0.08	0.14	8.8
5 hr.	0.5	51.4	21.4	(2 cells)	26.7	(1 cell)	0.19	0.13	9.4
Recovering larvae*	0.2	19.7	48.8	0.1	31.2	(1 cell)	0.06	0.22	3.6
Moribund larvae*	0.07	0.8	14.9	(1 cell)	84.1	0	0	(1 cell)	(4 cells)
Surviving larvae**	0.5	30.4	47.9	0.1	21.1	0	0.08	0.23	6.1
Carbon tetrachloride									
5 min.	0.8	33.6	31.0	0.3	34.2	0	0.10	0.13	12.2
2½ hr.	0.1	15.5	24.3	(2 cells)	60.1	0	0.12	0.07	3.2
5 hr.	0.4	23.5	31.7	(5 cells)	44.4	0	0.19	0.23	3.4
Recovering larvae*	0.1	15.4	44.2	0.1	40.2	0	0.23	0.25	3.4
Moribund larvae*	0	2.6	19.2	0	78.2	0	0	(1 cell)	(9 cells)
Methyl bromide									
5 min.	0.4	40.3	26.3	(2 cells)	32.9	(1 cell)	0.24	0.31	3.4
5 hr.	0.3	29.2	34.8	(4 cells)	35.7	(2 cells)	0.12	0.26	5.4
Recovering larvae*	0.3	31.8	58.2	0.1	9.6	0	0.10	0.20	10.1
Moribund larvae*	0.05	7.7	11.9	(1 cell)	80.3	0	0.17	0.12	1.7

* *48 hr. after fumigation.*

** *Successively treated with low concentrations during development (see text).*



Photomicrographs of haemocytes during and after fumigation with methyl bromide. (×1000)

FIG. 14. Group of haemocytes after five-minute exposure to fumigant, showing passive–active changes. FIG. 15. Group of haemocytes after 30-min. exposure to fumigant. Some cells show evidence of preliminary stages of cytolysis. FIG. 16. Group of haemocytes after two- and one-half-hour exposure to fumigant, showing agglutination and extensive degeneration. FIG. 17. Haemocytes after five-hour exposure to fumigant, showing extensive degeneration. FIG. 18. Haemocytes from a recovering larva 48 hr. after fumigation. FIG. 19. Haemocytes from a moribund larva 48 hr. after fumigation, showing complete breakdown.

cells of normal larvae just before pupation (1). Almost all of the spheroidocytes and a few of the plasmatocytes contained the lipoid, which also occurred to some extent as lipomicrons in the plasma. The cells of moribund insects contained only small amounts of the material. Evidently the presence of the lipoid in the spheroidocytes did not imply the onset of pupation since it also occurred in fourth- and fifth-instar larvae recovering from fumigation, although in smaller amounts. Histochemical tests showed that the lipoid was composed almost entirely of neutral fats.

Quantitative analysis of the blood lipoids gave the following results: (*a*) the fat content (ether-extractable fraction) of the blood of normal sixth-instar larvae was 10.4% of the dry weight, (*b*) the fat content of the blood of sixth-instar larvae recovering from fumigation at MLD_{50} of carbon tetrachloride was 14.9% of the dry weight. This is an increase of approximately 50% above the normal. A lesser increase (fat content 13.3% of the dry weight) was obtained in larvae subjected to MLD_{90} and sacrificed at the end of fumigation.

An increase in total body fat also occurred in larvae recovering from fumigation with carbon tetrachloride. The fat content of normal, untreated sixth-instar larvae was 37.4%. That of larvae recovering from fumigation with carbon tetrachloride was 44.7%, an increase of approximately 20% above the normal. Body fat increased about 15% above normal (43.1%) at the end of fumigation at MLD_{90}. A single test with fourth-instar larvae showed the normal body fat to comprise 27.0% of the dry weight, and that of recovering larvae 28.4%.

Discussion

It is significant that the haemocytes of larvae of *E. kühniella* responded in the same way to each of the three fumigants employed. Each of the fumigants was a halogenated organic compound, but one was an ether whereas the other two were hydrocarbons, and one was brominated whereas the other two were chlorinated. It is reasonable to suppose that the haemocytes would respond in much the same way to other organic fumigants. Similarity in response to a variety of ingested compounds was reported for the blood cells of the southern army worm (9).

With each compound, fumigation caused a decrease in total cell numbers. This was evident from the increased numbers of degenerate cells recorded in differential cell counts as well as from total cell counts. The decrease was very marked in larvae that were moribund 48 hr. after fumigation. Evidence of regeneration was usually present at the end of fumigation, and, in insects that recovered, substantial increases in cell numbers occurred during the 48-hr. recovery period. Normal numbers were not always attained in that time, although larvae recovering from carbon tetrachloride fumigation showed significantly greater than normal cell numbers. Furthermore, larvae that had been subjected to continuous fumigations with dichloroethyl ether during their development showed total cell numbers significantly greater than normal in the sixth stage. Evidently cell increases are associated with recovery.

Possibly the increases result from stimulation of cell division by injury substances as reported recently (3). Decreases in cell numbers following the administration of poisons and their subsequent increase have been reported for various species (2, 4, 5, and 9).

The response of the haemocytes to the fumigants progressed through three phases similar to those reported for the southern army worm (9). They are: (*a*) physiological changes involving passive–active transformations, (*b*) pathological changes resulting in cell breakdown and decrease in cell numbers, and (*c*) regenerative changes involving an increase in mitotic activity and resulting in an increase in cell numbers, particularly of the spheroidocytes. The regenerative phase included the appearance of globules of neutral fats in the spheroidocytes. Failure to recover was accompanied by almost complete destruction of the haemocytes before death.

The immediate passive–active change of the cells implies a reaction to the toxic material, but the function, if any, is obscure. Although this action is normally reversible, it was followed here by agglutination and necrosis. Agglutination was an early feature of pathological change presumably associated with changes in the cell membrane; possibly it resulted from the fat-solvent properties of the fumigants. Agglutination differed somewhat in the two major cell classes. The plasmatocytes clotted together in a mass, often encompassing other cell types. The spheroidocytes in the early stages of agglutination tended to adhere at points on their periphery and form a ring of connected cells. This further distinguishes the major classes.

Further distinctions in characteristics of the cell classes were evident from their sensitivity to the fumigants. The prohaemocytes were seldom affected at the concentrations used. The plasmatocytes reacted quickly and underwent rapid cytoplasmic and nuclear breakdown. The spheroidocytes were less quickly affected by the fumigants but gradually became eosinophilic and ruptured, or quickly became misshapen and underwent cytolysis. Their nuclei retained their form for some time after complete cytoplasmic breakdown.

The increase in the total number of cells in larvae recovering from fumigation was largely the result of spheroidocyte increases. This was shown by differential cell counts. Their increase was apparently largely the result of increased mitotic activity. The plasmatocytes increased, but not to the same extent. Presumably, the increase in numbers of one particular class of cells implies some function peculiar to the conditions that caused it. In this case, presumably, the spheroidocytes are associated with recovery from the effects of the fumigants. This class of cells was found to be associated with recovery from other abnormal conditions (1). In the present situation their numerical increase was accompanied by the appearance of intracellular lipoids in large amounts. A similar condition was reported (1) for these cells just before pupation in normal insects. Apparently this condition does not represent

fatty degeneration, since the cells appear normal in other respects, it seldom occurred in other cell classes, and it was a normal occurrence just before pupation. The lipoid evidently was not derived directly from ingested food, since excessive feeding did not occur during recovery or just before pupation. Although it is possible that the fumigants, each a fat solvent, may be directly responsible for the appearance of the lipoids, it seems very unlikely that such is the case here since the appearance of intracellular lipoids in these cells is a normal occurrence before pupation without the interference of fumigants. Furthermore, it did not appear to any great extent during fumigation, but increased in larvae recovering from the toxicants. Increase in blood lipoids and in total body fat was greater in insects recovering from fumigation after 48 hr. than in insects subjected to a high concentration of the gas and not allowed a recovery period. Evidently the release of fats by the solvent action of the fumigant is less than that produced during recovery from a smaller amount of fumigant. If the lipoid released by solution in the fumigant is not considered, the lipoid may be accounted for in two ways. Either it represents fats that have been released from a bound and undemonstratable condition in the spheroidocytes, or it results from synthesis from carbohydrates or proteins. The latter appears the most likely condition. Munson and Yeager (4) have reported the appearance of fats in the haemocytes of the southern army worm, *Prodenia eridania* (Cram.), as glycogen disappeared from them. Apparently the spheroidocytes are active in fat metabolism and in this regard, at least, may be important in recovery from conditions of stress through the mobilization of energy reserves.

The results of these experiments do not clearly resolve the role of the haemocytes in the defence of the insect against toxicants. They do suggest, however, that these cells, particularly the spheroidocytes, may contribute to recovery from the effects of fumigants, possibly through their apparent ability to create fat reserves. Too much speculation is unwise at this time, but it seems possible that the appearance of lipoids in these cells just before pupation and during recovery from the effects of fumigants are analogous and concerned with effective utilization of reserves during periods of decreased respiratory metabolism.

Conclusions

The haemocytes of the Mediterranean flour moth, *Ephestia kühniella* Zell., are associated with the recovery of this species from the effects of three organic fumigants. Presumably their activities would be similar with other organic compounds. The haemocytes are seriously affected during fumigation and total cell numbers drop to a low level. This decrease continues in individuals that fail to recover from the effects of the fumigant, and their numbers are negligible 48 hr. after fumigation when larvae are in a moribund condition. Recovery from the effects of the fumigants is accompanied by rapid increase in cell numbers above the normal total. The increase is largely on the part of the spheroidocytes. During their numerical increase the spheroidocytes contain relatively large amounts of intracellular lipoids. At the same time there

is a significant increase in amounts of ether-extractable blood lipoids and total body fats. It is suggested that the spheroidocytes are important in recovery through their apparent ability to synthesize fats from other materials and thus contribute to the available energy reserves.

Acknowledgments

The writer wishes to thank Dr. A. W. A. Brown, Head of the Department of Zoology, University of Western Ontario, London, for his interest and counsel in directing this research. The investigation was financially assisted by a scholarship from the Agricultural Institute of Canada.

References

1. ARNOLD, J. W. The haemocytes of the Mediterranean flour moth, *Ephestia kühniella* Zell. (Lepidoptera: Pyralididae). Can. J. Zool. 30 : 352–364. 1952.
2. FISHER, E. A. The effect of a few toxic substances on the total blood cell count in the cockroach, *Blatta orientalis*. Ann. Entomol. Soc. Am. 29 : 335–340. 1936.
3. HARDING, D. Initiation of cell division in the *Arbacia* egg by injury substances. Physiol. Zool. 24 : 54–67. 1951.
4. Munson, S. C. and Yeager, J. F. Fat inclusions in the blood cells of the southern armyworm (*Prodenia eridania*). Ann. Entomol. Soc. Am. 37 : 396–400. 1944.
5. PILAT, M. The effects of intestinal poisoning on the blood of locusts. Bull. Entomol. Research, 26 : 283–292. 1935.
6. SHULL, W. E., RILEY, M. K., and RICHARDSON, C. H. Some effects of certain toxic gases on the blood of the cockroach, *Periplaneta americana*. J. Econ. Entomol. 25 : 1070–1072. 1932.
7. STRAND, A. L. Measuring the toxicity of insect fumigants. Ind. Eng. Chem. 2 : 4–8. 1930.
8. YEAGER, J. F., MCGOVRAN, E. R., MUNSON, S. C., and MAYER, E. L. Effect of blocking haemocytes with Chinese ink and staining nephrocytes with trypan blue upon the resistance of the cockroach, *Periplaneta americana* to sodium arsenite and nicotine. Ann. Entomol. Soc. Am. 35 : 23–40. 1942.
9. YEAGER, J. F. and MUNSON, S. C. Changes induced in the blood cells of the southern armyworm, *Prodenia eridania* by the administration of poisons. J. Agr. Research, 64 : 307–332. 1942.

THE NONMUTAGENIC ACTION OF *p-p'*-DDT AND γ-HEXACHLOROCYCLOHEXANE IN *DROSOPHILA MELANOGASTER* MEIG. (DIPTERA: DROSOPHILIDAE)[1]

By D. P. Pielou[2]

Abstract

As experiments are in progress on selective breeding for insecticide tolerance in insect parasites and as various physiologically potent chemicals have been shown to cause mutation in *Drosophila melanogaster* Meig., tests were made to determine whether *p-p'*-DDT and γ-hexachlorocyclohexane had any mutagenic action. Wild type males of *D. melanogaster* that had been exposed to the former insecticide in their larval feeding medium, or, as adults, to the vapor of the latter insecticide, were tested. The Muller-5 method of detecting recessive lethal mutations in the X chromosome was used. No evidence of mutagenic action was found in either insecticidal isomer.

Introduction

At the Belleville laboratory studies are being carried out on the selection of beneficial insect parasites for resistance to insecticides (Pielou and Glasser (7, 8)). It was thought desirable to check for mutagenic action in the insecticides because a variety of organic chemicals have been shown to cause mutation in *Drosophila melanogaster* Meig. These include, for instance, the vapors of mustard gases (Auerbach and Robson (3), Auerbach (1)), various carcinogens administered as aerosols (Demerec (4)), and formaldehyde when incorporated in the larval food medium (Rapoport (9), Kaplan (6)). The tests were considered a necessary preliminary in the Belleville work because the action of a physical agency that was mutagenic as well as selective would be complex and would need unusual treatment.

Method of Exposure to Insecticides

The supposition is that a chemical mutagen must reach the gonads to be effective. Gaseous or aerosol mutagens presumably enter through the spiracles and reach the gonads through the tracheae present in them (4).

DDT has no fumigant action and cannot be tested as a vapor. Instead it was mixed with the larval food medium so that the insects were exposed to the material for most of their larval life; it was assumed that the DDT would reach the developing gonadial tissue. Formaldehyde has been shown to be mutagenic when used in this way (9, 6), though the action here is indirect and is through some new substance formed by interaction with the food medium (2).

Pure parapara isomer of DDT (m.p. > 108° C.) dissolved as a 1% solution in ethanol was added to the standard corn meal – molasses–agar–yeast culture medium in the preparation stage. An amount of insecticide sufficient to

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make a medium with 20 parts per million of DDT by weight was added. This medium gave approximately a 50% reduction of emergent population as compared with controls. Previously it had been found that a medium with 5 p.p.m. of DDT, which has a marked effect on some stocks (Kalina (5)), has little effect on the stock of wild type *D. melanogaster* that has been reared in the Belleville laboratory for some years and was used in the experiments. Six pairs of these flies were put in each half-pint culture bottle, and males of the succeeding generation, of which the larvae had lived in the DDT medium, were tested for induced mutations.

In the experiments with γ-hexachlorocyclohexane (γ benzene hexachloride) advantage was taken of the fumigant action of the material. With a gaseous mutagen the adult insect may be exposed (3, 4) with a reasonable hope that the material will reach the gonads via the tracheae. Crystals of lindane (> 99% pure γ isomer of hexachlorocyclohexane) were placed in a Syracuse watch glass which was then covered with a slightly larger filter paper. About 50 young males, anaesthetized with ether, were then placed on the filter paper and covered with a second inverted watch glass. In this small chamber they were exposed to the vapor of the crystals. They were exposed for about 17 min. but for most of this period they were in the anaesthetized state; for the last minute or so they were intensely active on recovering from the ether. The mortality caused by the vapor varied greatly and was 100% in several cases. Mutation tests were carried out on insects that survived exposures in which approximately 50% mortality was recorded.

Method of Detecting Mutation

The Muller-5 method of detecting recessive lethal mutations in the female-determining X-chromosome of the sperms of the treated males was used. As most mutations are recessive lethals this test will disclose any general mutagenic tendency of material that is being examined. This test is very similar to the older C l B test. Each treated wild type male was mated to a virgin female of Muller-5 stock. In the latter stock the X chromosome possesses a double inversion, which suppresses crossing over. This chromosome also carries the genes for *apricot eye* and *Bar eye*, which act as markers and also allow ready distinction of the homozygous-X and heterozygous-X states. This distinction among offspring allows a later check on the virginity of Muller-5 mothers before their matings to treated wild type males. The daughters of these matings, which are heterozygous for the Muller-5 X chromosome, carry any induced recessive lethal in their other, wild type, chromosome. These daughers are individually mated as virgins to young Muller-5 males (hemizygous for the Muller-5 X chromosome). The sons of these matings have each derived their single X chromosomes from their mothers. If the wild type X chromosome carries a lethal mutation then sons with the wild type eye will be absent; only male offspring with apricot Bar eye will be present. (If no mutation has occurred both types will be present.)

This is accepted as evidence of mutation in the sperm of the originally treated grandfather. It is, however, checked through another generation by crossing virgin heterozygous-X daughters of the suspect culture to Muller-5 males and again confirming the absence of sons with the wild type eye.

Results

The results obtained are summarized in Table I, There is clearly no evidence of the induction of recessive lethal mutations in the X chromosome by the necessarily short exposures to the highly toxic γ-hexachlorocyclohexane vapor or by lengthy larval exposures to a low concentration of DDT in the food. With most of the chemical mutagens studied (1) there is no difficulty in obtaining lethal mutation rates of 1% to over 10% and a few minutes' total exposure is all that is required with such mutagens as the nitrogen and sulphur mustards.

TABLE I

Treatment	Number of cultures equivalent to "sperm" tested"	Number of lethal mutations in X chromosomes	Percentage lethal
γ-hexachlorocyclohexane	961	2	0.21
p-p'-DDT	1251	2	0.16
Control	1570	3	0.19

References

1. AUERBACH, C. Chemical mutagenesis. Biol. Revs. Cambridge Phil. Soc. 24 : 355–391. 1949.
2. AUERBACH, C. The mutagenic mode of action of formalin. Science, 110 : 419–420. 1949.
3. AUERBACH, C. and ROBSON, J. M. The production of mutations by chemical substances. Proc. Roy. Soc. Edinburgh, B, 62 : 271–283. 1947.
4. DEMEREC, M., WITKIN, E. M., NEWCOMBE, H. B., and BEALE, G. H. The gene. *In* Carnegie Inst. Wash. Year Book, 46 : 127–135. 1947.
5. KALINA, B. F. Development and viability of *Drosophila melanogaster* on a medium containing DDT. Science, 111 : 39–40. 1950.
6. KAPLAN, W. D. Formaldehyde as a mutagen in *Drosophila*. Science, 108 : 43. 1948.
7. PIELOU, D. P. and GLASSER, R. F. Selection for DDT tolerance in a beneficial parasite, *Macrocentrus ancylivorus* Roh. I. Can. J. Zool. 29 : 90–101. 1951.
8. PIELOU, D. P. and GLASSER, R. F. Selection for DDT resistance in a beneficial insect parasite. Science, 115 : 117–118. 1952.
9. RAPOPORT, I. A. Carbonyl compounds and the chemical mechanism of mutation. Compt. rend. acad. sci. U.R.S.S. 54 (n.s.) : 65–67. 1946.

OBSERVATIONS ON THE DEVELOPMENT OF *LYMNAEA PALUSTRIS* MÜLLER[1]

By Bruce M. McCraw[2]

Abstract

Observations were made in the laboratory on the egg-laying habits, egg masses, and embryos of isolated *Lymnaea palustris* Müller placed in equal volumes of water (415 cc.). It was found that the mean number of eggs per mass decreased as *L. palustris* oviposited more frequently, but that the mean percentage of embryos surviving to emerge from the masses increased. This inverse relation between egg production and survival of embryos resulted in the hatching of a mean number of embryos which was approximately constant for each of a series of ovipositions. The factors which may determine or influence this relation were discussed (amount of dissolved oxygen, temperature, volume and pH of the water, nutrition, and disease). Factors similar to those affecting the number of surviving embryos probably influenced the rate of decrement of egg production. Most of the egg masses were laid on a horizontal plane, suggesting that a balance mechanism operates, the snail showing preference for this position during egg deposition.

Introduction

Lymnaea palustris is an important intermediate host for the liver fluke *Fasciola hepatica* Linnaeus, 1758, and probably also *Fascioloides magna* (Bassi, 1875) which has been reported (5) recently in Cervidae from 14 counties or districts in Ontario. Consequently, a knowledge of the ecology of this and certain other aquatic snails is important in the study of the spread of these trematodes from host to host. Population density of aquatic snails may influence the rate of spread and extent to which domestic as well as wild ruminants and other animals are infested with trematode parasites. It would, therefore, be desirable to investigate the various factors that influence the reproductive potential, and the rise and fall in numbers of aquatic snails, especially those most important in parasitology. These studies were undertaken, in part, to learn some of the factors which may reflect the behavior of *L. palustris* in the field.

Experience in rearing aquatic snails in the laboratory is also necessary in life cycle studies of flukes. Krull (6) emphasized this and gave valuable information on raising *Gyraulus parvus* (Say) in the laboratory. The parasitologist may find it necessary to maintain large numbers of snails in isolation and under those circumstances conservation of space becomes a problem. If individual snails can be raised in relatively small volumes of water then further information would be desirable on the survival times of those snails. Such data may determine the duration of time required for an experiment to be carried out and may decide the initial number of snails required to allow for mortalities and at the same time still have enough survival to obtain satisfactory results.

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Materials and Methods

Twenty-six *L. palustris*, collected from a nearby pond and matched as closely as possible for size, were placed in jars (one to a jar) containing 415 cc. of tap water. The inside diameter of these containers was 7.5 cm. and the height 10.5 cm. The water was changed twice daily, when the snails were fed and when the uneaten lettuce was removed. No other material was added to the jars containing adult *Lymnaea*. A cover, which permitted the entrance of air, was placed on each container to prevent snails from escaping. When the water was first poured into containers, its temperature was 10.5° to 11° C. and gradually warmed up in a few hours to about 23° to 24° C. The pH was 6.9. Snails were not exposed to sunlight and light brown paper was placed under the containers. Dead *Lymnaea* were replaced by others of similar size in clean jars which should always be used to prevent the transmission of possible pathogenic bacteria and fungi to the new snails.

As egg masses were counted, the parent snail was removed and placed in a new container. Clean water (415 cc.) was added to the old jar containing the egg mass but this was not changed again, only replenished as evaporation took place. A cover was placed on the jar as it was on the jars containing the parent snails.

For each snail, records were kept of the date of oviposition, the number of egg masses, the size of egg masses (length and width in millimeters), the number of eggs per mass, and the position and shape of masses in the containers. Several egg counts for each mass were made to ensure accuracy. Observation was also made of the time, in days after laying, that emergence of embryos from a mass began and ended (hatching interval), as well as of the number and percentage of embryos that survived. Survival of embryos was expressed as the percentage of embryos in each mass that emerged. Death of embryos was determined by lack of movement and evident development and frequently by a clouding of the egg mass possibly caused by fungus growth or certain water-inhabiting bacteria. The egg-laying interval was the time between the deposition of individual egg masses, and the hatching time the number of days between oviposition and the time when the first embryo was seen to emerge.

Results and Discussion

Only 10 snails survived through the entire period of observation (63 days). The mean survival time of *L. palustris* was a little over 31 days (Table II). Of a total of 51 snails used, 29 produced eggs. Amongst ovipositing snails that died, death occurred in the majority within seven days after the last oviposition. No perceptible increase in shell size of parent snails was noted throughout the period of observation.

There is a gradual decrease (Fig. 1) in the mean number of eggs per mass as *L. palustris* oviposits more frequently. Bondesen (1) recorded that there was a decline in egg production towards the close of the breeding season in members of the Lymnaeidae and more gradually in the Planorbidae and Ancylidae.

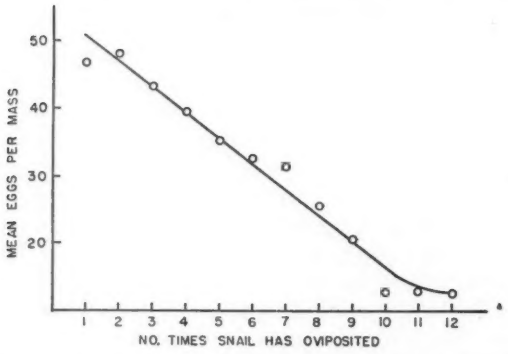

FIG. 1. The relation between the number of ovipositions and the number of eggs per mass.

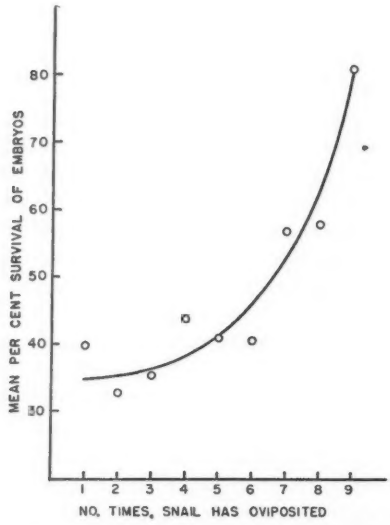

FIG. 2. The relation between the number of ovipositions and the percentage survival of embryos.

Correlated with this decrement is an increase in the mean percentage of embryos which survive to emerge from the egg masses (Fig. 2). This inverse relation between egg production and survival of embryos (Fig. 3) results in the hatching of a mean number of embryos which approaches a constant value for each of a series of ovipositions by adult *L. palustris* (Table I). Although the factors which govern the number of embryos which emerge from an egg mass are not definitely known, it may be suggested that the amount of oxygen available to the developing embryos is one of the more important factors. The egg masses were uniform in width (most of them were 3 mm. wide) but

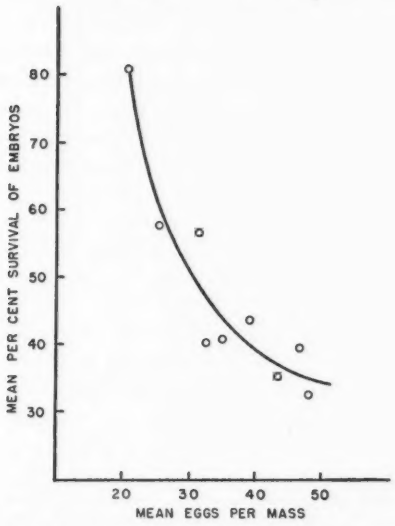

FIG. 3. The relation between the number of eggs per mass and the percentage survival of embryos.

they varied considerably in length, depending on the number of embryos within them (maximum 23.5 mm., 82 embryos). Since width was a constant feature, the oxygen demand of the individual mass would be a function of length or the number of embryos within it, and temperature, as it may affect oxygen requirements. Table I shows, for each of a series of ovipositions, that the mean number of surviving embryos corresponds closely to the product of the mean number of eggs per mass and the mean percentage survival of embryos, suggesting that the number of embryos surviving to hatching follows a relation whereby

$$E_m S_m = \text{a constant}$$

for a given temperature and amount of dissolved oxygen,
where

E_m = mean number of eggs per mass

and

S_m = mean percentage survival of embryos.

While it is suggested that the amount of dissolved oxygen and temperature are the important determining factors, the interaction of several other factors may influence the number of hatching embryos. Amongst them are the volume and pH of the water, nutritional factors, and disease. In the natural habitat of *L. palustris* the presence of other invertebrates or aquatic vertebrates and predators would provide additional factors.

The rise in the percentage survival of embryos may also be partly due to an increased viability of embryos with the age of the parent snail. This

TABLE I

EGG PRODUCTION AND SURVIVAL OF EMBRYOS OF *Lymnaea palustris*

Oviposition	Mean number of eggs per mass (E_m)	Mean % survival of embryos (S_m)	E_mS_m	Mean survival of embryos
1	46.7	39.6	18.6	17.5
2	48.0	32.6	15.7	14.7
3	43.1	35.2	15.1	16.0
4	39.1	43.7	17.0	19.4
5	35.1	40.9	14.3	14.5
6	32.6	40.3	13.3	11.4
7	31.3	56.7	17.6	19.0
8	25.5	57.8	15.0	11.0
9	20.3	80.1	16.0	21.0
		Total	142.6	144.5
		Mean	15.8 (16)	16.1 (16)

possible effect of age of *L. palustris* on the viability of embryos could be studied by adjusting the volume of water so that the amount per embryo is the same.

The mean percentage mortality of all embryos was fairly high (Table II, 56.9%) probably because the water containing the developing embryos was not aerated. Noland and Carriker (7) found that insufficiently aerated egg masses of *Lymnaea stagnalis appressa* Say did not hatch.

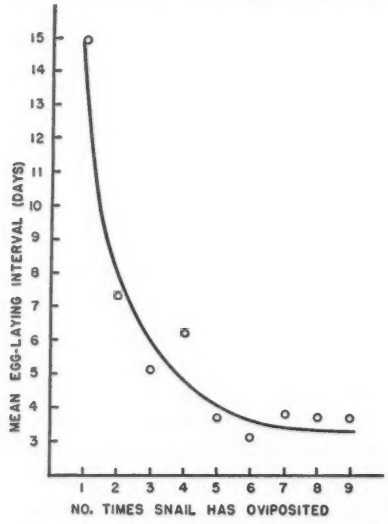

FIG. 4. The relation between the number of ovipositions and the egg-laying interval.

Factors similar to those affecting the number of surviving embryos probably also influence the rate of decrease of egg production (amount of dissolved oxygen, temperature and pH of the water, nutrition, and disease). In addition, the absence of cross-fertilization, senility, and genetic factors may affect egg production. Noland and Carriker (7) found for *L. s. appressa* that egg-laying could be approximately doubled by the addition of cooked wheat cereal to the normal lettuce diet. Conversely, when the wheat cereal was discontinued in the diet egg production decreased. A liver diet favored egg production only slightly. Forbes (4) observed that small amounts of vitamin B_1 added to the standard diet of *Lymnaea columella* Say exerted a stimulating effect on fertility, but that larger amounts retarded this function. There is also evidence that isolated noncopulating snails (*L. s. appressa*, *L. columella*) lay more eggs than paired or grouped snails (7, 9). It may be that certain strains of *L. palustris* show different rates of decrease in the number of eggs per mass with age.

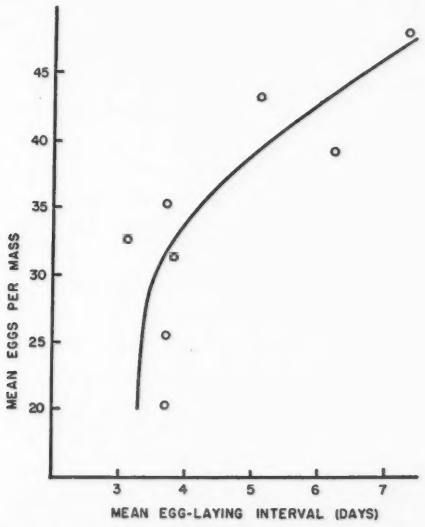

FIG. 5. The relation between the egg-laying interval and the number of eggs per mass.

Gradual decrease in the number of eggs per mass may also point to a cycle in egg-laying. Crabb (3) found that isolated laboratory stock of *L. s. appressa* deposited eggs about every third day for a period of about 25 days, rested a few days, and then began another cycle of laying. Six of my isolated snails had oviposited more or less continually for 45 days or more, at which time there was no evidence of a rest period. In each case the last egg-laying interval still averaged only a little over five days although the average number of eggs for the final masses was, however, well below the over-all mean (36.2, Table II).

TABLE II

DATA ON THE BEHAVIOR OF ADULTS, EGG MASSES, AND EMBRYOS OF *Lymnaea palustris*

	Mean	Range	
Survival time of *Lymnaea palustris* (days)	31.3	—	—
Egg-laying interval (days)*	4.8	19	**
Hatching time (days)	10	15	5
Hatching interval (days)	10.4	15	7
Eggs per mass	36.2	82	1
Percentage mortality of embryos	56.9	100	0

* *These values are not based on the first interval, which was exceptionally long for most snails. See text.*

** *Occasionally more than one egg mass was laid in one day.*

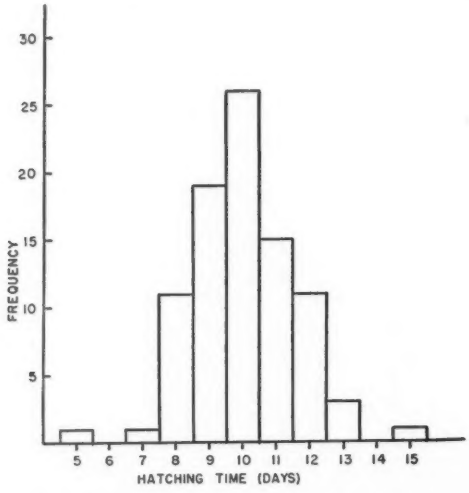

FIG. 6. The frequency of hatching times.

Decrease in the egg-laying interval with age (Fig. 4) is probably due to the gradual production of fewer eggs per mass (Fig. 5). The interval leveled off (Fig. 4) after the fourth egg deposition. There was often a considerable lapse of time before the appearance of the first egg mass, the majority not appearing before the 14th to the 17th day. The mean of the intervals before the first oviposition was 15 days. This lapse of time may be a period of acclimation as a result of a change of environment from pond to aquaria or a period necessary for the development of eggs within the parent. Table II shows the mean and range of the egg-laying intervals. The hatching time of 68% of the egg masses (Fig. 6) was from nine to 11 days with the highest frequency and mean at 10 days (Table II).

Of the total number of egg masses laid, 63% were found on the bottoms of the jars, 35% on the sides, and 2% were laid in corners, partly on the side and partly on the bottom. This suggests that a balance mechanism operates, the snail showing preference for this position during the egg deposition. The area of the wall of each container covered by water was nearly five times greater than the bottom showing further that selection of this plane for oviposition was more than the result of chance alone. Noland and Carriker (7) stated that there is a tendency for egg masses of *L. s. appressa* to be affixed to surfaces away from light and of darker coloration. However, the effect of variation of light on ovipositing *L. palustris* was reduced to a minimum by placing light brown paper under all aquaria so that the bottoms of the jars were nearly as light as the sides. It would seem, therefore, that a balance mechanism determines the position for egg-laying, and the findings of Noland and Carriker (7) would suggest that variation in light intensity influences the location for egg deposition. *L. palustris* oviposited infrequently during the day indicating that light also influences the time for egg-laying. The question of whether the balance mechanism dominates over light as it affects location probably varies with environmental conditions. It is conceivable that the light factor may influence the snail to oviposit in a position which it might not otherwise select. However, in the present experiment, it was found that when variation in light intensity was reduced in aquaria the snail chose most frequently a horizontal plane for oviposition.

It was not found necessary to provide lettuce or other material for the parent *Lymnaea* to lay eggs upon. Furthermore, after a few days lettuce decomposes and the resultant contamination of water is deleterious both to the adult snails and to the developing embryos. The egg masses adhered well to glass and were difficult to remove; scraping was frequently necessary unless the masses or embryos were diseased.

The adult *Lymnaea* gradually became used to being fed; they soon found lettuce placed in aquaria and began feeding. If placed on a leaf of lettuce they extended the foot almost immediately in preparation for eating. When all snails were feeding an audible rasping of the radula was heard.

A great number of the recently hatched snails crept up the sides of the containers and remained there, many of them dying as a result of desiccation. Several authors have observed this behavior in snails closely related to *L. palustris* (8, 2, 9). This can present a serious problem in rearing young snails but can be alleviated somewhat by raising the level of the water to near the top of aquaria. Noland and Carriker (7) indicated that this procedure is related to the freeing of the respiratory chamber of fluid.

No relation was found between the egg-laying interval and the percentage survival of embryos, between the hatching interval and the percentage survival of embryos, or between the number of times the snail had oviposited and the hatching interval. Further investigation, however, may reveal some relation between these characteristics.

Acknowledgments

I wish to express my appreciation to Dr. Henry van der Schalie of the Department of Zoology of the University of Michigan for his many helpful suggestions and reading of the manuscript.

References

1. BONDESEN, Poul. A comparative morphological-biological analysis of the egg capsules of fresh water pulmonate gastropods. Naturhistorisk Museum, Aarhus, Denmark. Reprinted from Natura Jutlandica, 3 : 1–208. 1950.
2. COLTON, H. S. Some effects of environment on the growth of *Lymnaea columella* Say. Proc. Acad. Nat. Sci. Phila. 60 : 410–448. 1908.
3. CRABB, E. D. The fertilization process in the snail, *Lymnaea stagnalis appressa* Say. Biol. Bull. 53 : 67–106. 1927.
4. FORBES, G. S. The effect of added amounts of vitamin B_1 on the life cycle of *Lymnaea columella*. Anat. Record, 84 : 526. 1942.
5. KINGSCOTE, A. A. The significance of the large American liver fluke, *Fascioloides magna* (Bassi, 1875) in deer, with particular reference to its distribution in Ontario. Paper given in Kingston, Ontario, at the Technical Session of the Fish and Wildlife Advisory Committee. February, 1950.
6. KRULL, W. H. Importance of laboratory-raised snails in helminthology with life history notes on *Gyraulus parvus*. Occasional Papers Museum Zool. Univ. Mich. 226 : 1–10. 1931.
7. NOLAND, L. E. and CARRIKER, M. R. Observations on the biology of the snail *Lymnaea stagnalis appressa* during twenty generations in laboratory culture. Am. Midland Naturalist, 36 : 467–493. 1946.
8. WALTER, H. E. Behavior of the pond snail *Lymnaeus elodes* Say. Cold Spring Harbor Monographs, 6 : 1–35. 1906.
9. WINDSOR, C. P. and WINDSOR, A. A. Longevity and fertility in the pond snail, *Lymnaea columella*. J. Wash. Acad. Sci. 25 : 302–307. 1935.

THE EARLY DEVELOPMENT OF A BOTTOM FAUNA IN A NEW POWER RESERVOIR IN THE ROCKY MOUNTAINS OF ALBERTA[1]

By J. R. Nursall[2]

Abstract

The Barrier reservoir, Alberta, was examined periodically from May, 1947, shortly after water impoundment began, until June 1949. The reservoir is fundamentally oligotrophic; rapid replacement of water, periodic fluctuation of the water level, and a marked annual deposition of sediment contribute to this condition. The bottom fauna, consisting chiefly of immature Chironomidae, was much influenced by these factors during the period of the investigation. A *Pentapedilum*-Chironomus-Tanytarsus succession is interpreted as indicating a change from original eutrophic conditions on the bottom to oligotrophic, as the rich leaf litter of the original bottom was lost under sediments.

Introduction

The creation of the Barrier reservoir on the Kananaskis river, Alberta, presented an exceptional opportunity to study the biological sequence within a new body of water. The special study of the macroscopic bottom fauna of the Barrier reservoir was one phase of a larger investigation undertaken during 1947, the aim of which was to examine the effects on fish of power plant and reservoir developments long the Bow and Kananaskis river systems (8).

The Barrier reservoir supplies water to a power plant established by the Calgary Power Co. in 1947. Impoundment of water within the reservoir began during May, 1947, and by the end of that year the water level approximated full supply level.

Data and samples were collected from the Barrier reservoir semimonthly from June 1 to Sept. 15, 1947. Further examinations were made during January and June of 1948 and in the same months of 1949. In addition to the bottom fauna, the physicochemical conditions and the plankton were studied.

Methods and Apparatus

The oxygen content of the water was measured by Miller's method; pH was determined by using phenol red in a Hellige comparator. During 1947, the temperature was recorded by bathythermograph and reversing thermometer. The thermometer alone was used during 1948 and 1949; trouble was experienced with this instrument during the examinations of 1949 so the record of temperatures is incomplete. The plankton samples were collected with a net of the Wisconsin type, of No. 20 bolting silk, and with a mouth diameter of 25 cm.

Bottom sampling was first attempted with an Ekman dredge covering an area of 500 sq. cm. The leaf litter covering the floor of the reservoir prevented

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the jaws of the dredge from closing tightly so a heavier Petersen dredge was substituted. This dredge covered an area of 662.25 sq. cm. The Petersen dredge was used for all examinations subsequent to July, 1947. In June, 1949, the Petersen dredge also was used to bring up a nearly undisturbed segment of the bottom sediments. This was accomplished by carefully opening the dredge over a large, flat pan so that the enclosed sample was laid out in a natural position. In this way close examination of the layers of sediment was possible and the distribution of the bottom fauna within the mud could be noted.

A core sampler (Fig. 1), modified from a design described by Welch (14), was used during January and June, 1949, to investigate the sediments of the reservoir. Essentially, the sampler consists of $2\frac{1}{4}$ in. brass tubing, 22 in. in length; about 8 lb. of lead is molded around the top portion of the tube, to act as a weight to drive the sampler into the mud. The cutting edge of the sampler is of brass and screws into the lower end of the tube as a nosecap.

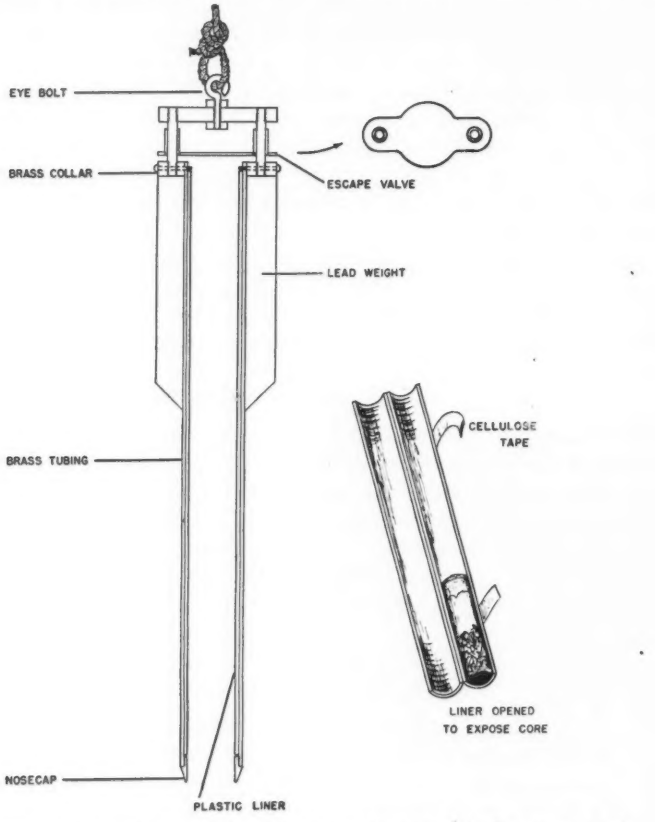

FIG. 1. Core sampler used to study the sedimentary deposits of the Barrier reservoir.

A brass collar surrounds the tube at the upper end to support two upright posts, which guide a free-sliding escape valve and support the cable attachment. The liner of the sampler is plastic tubing held in place at the upper end by the screws attaching the collar to the brass tube, and at the lower end by the nose-cap. The liner is sawn in half lengthwise and bound together with cellulose tape. For examination, the liner is opened to bare the core. During the investigation all cores were examined as soon as collected. Use of this instrument proved satisfactory except during winter when icing reduced its efficiency. It is believed that little distortion due to compression occurred, the diameter of the tube being large enough to minimize this.

Morphometry

The Barrier reservoir is formed by the Kananaskis river valley, about eight miles upstream from the junction of the Kananaskis and Bow rivers. This junction is 40 miles west of Calgary, Alta.

At the site selected for the dam, the Kananaskis river flows through a gorge 130 ft. deep and several hundred yards long. The gorge is most narrow at the upstream end; here the dam was constructed. The dam is an earth-fill structure of clay. It is about 1600 ft. in length and the greatest width of the base is more than 600 ft. The reservoir face of the dam is riprapped with large boulders.

Upstream from the dam, the river valley is broad and flat for several miles. The area to be flooded was cleared of timber to a level about five feet above the intended full supply level. The result was a basin covered with leaf litter, shrubby vegetation, and short stumps where timber had been standing.

The Kananaskis river has a drainage area of approximately 360 square miles. Of this area, at least 330 square miles contribute their runoff to the reservoir. The remaining 30 square miles drain into the river downstream from the dam.

Fig. 2 shows the outline and depth contours of the Barrier reservoir. The contours have been plotted from a pre-impoundment topographical map of the reservoir basin supplemented by extensive soundings.

At full supply level the elevation of the reservoir surface is 4515 ft. (1375 m.) above sea level. The total length of the reservoir is 2.25 miles (3.62 km.), which is made up of two arms forming nearly a right angle around the base of Barrier mountain. The maximum breadth of the reservoir is 2100 ft. (0.64 km.). The surface covers 761 ac. (308 ha.). The shoreline is 10.5 miles (16.9 km.) in length; the shore development is 2.25. The volume of water which can be stored within the reservoir is approximately 1661 million cubic feet (47.3 million cubic meters) (Table I).

Throughout the greater part of the reservoir the bottom is comparatively flat adjacent to the old river bed. However, the walls of the basin are generally steep with some slopes approaching 40°. Only at the extreme ends of the reservoir and along the north shore are there gradual slopes where a littoral region can be defined. Such gently-sloping areas make up nearly 40% of

the shore length, but because of water level fluctuation these shallow areas can contribute little to the organic production of the reservoir. In general, the reservoir presents a broad U-shape in cross section.

Table I presents hydrographic data for the reservoir at full supply level. Table II shows the breakdown of these data for each of the two arms. Henceforth, to simplify the discussion, the upstream or north–south arm of the reservoir will be referred to as region I and the downstream or east–west arm will be referred to as region II.

Calculations based on the data of Table II show that region II contains 84% of the volume of water and 64% of the surface area of the reservoir. These data are reflected in the mean depths of the two regions. The mean depth of region I is 6.9 m., while that of region II is 20.1 m. The mean depth of the reservoir as a whole is 15.4 m. Region I is smaller and shallower, but, as will be shown, is subject to more variable physical conditions than is region II. These physical conditions tend to deprive region I of much of its potential productivity and the benefits that could accrue from this.

FIG. 2. Hydrographic map of the Barrier reservoir.

TABLE I

HYDROGRAPHIC DATA FOR THE BARRIER RESERVOIR AT FULL SUPPLY LEVEL

Depth, m.	Area		% Total area	Stratum, m.	Area between contours		% Total area	Volume cu.m. $\times 10^6$	% Total volume
	Acres	Ha.			Acres	Ha.			
0	761	308	100.0	0–5	173	70	22.7	13.65	28.8
5	588	238	77.3	5–10	110	44	14.3	10.80	22.8
10	478	194	63.0	10–15	127	52	16.9	8.40	17.7
15	351	142	46.1	15–20	69	28	9.1	6.40	13.5
20	282	114	37.0	20–25	98	39	12.8	4.73	10.0
25	184	75	24.3	25–30	132	54	17.4	2.40	5.1
30	52	21	6.8	30–39	52	21	6.8	0.95	2.1
		Totals			761	308	100.0	47.33	100.0

TABLE II

COMPARISON OF HYDROGRAPHIC FEATURES OF REGIONS I AND II OF THE BARRIER RESERVOIR AT FULL SUPPLY LEVEL

Stratum, m.	Region I				Region II			
	% Situated in region I	Area		Volume, cu.m. $\times 10^6$	% Situated in region II	Area		Volume, cu.m. $\times 10^6$
		Acres	Ha.			Acres	Ha.	
0–5	71.0	123	50	4.25	29.0	50	20	9.40
5–10	68.6	75	30	2.25	31.4	35	14	8.55
10–15	46.6	59	24	0.90	53.4	68	28	7.50
15–20	21.3	15	6	0.15	78.7	54	22	6.25
20–25	0.0	—	—	—	100.0	98	39	4.73
25–30	0.0	—	—	—	100.0	132	54	2.40
30–39	0.0	—	—	—	100.0	52	21	0.95
Totals		272	110	7.55		489	198	39.78

Seasonal Fluctuation of Level

The morphometry of the reservoir was treated conventionally in the preceding paragraphs. However, the seasonal fluctuation of the water level subjects the reservoir to conditions which exert a profound influence on this body of water.

The Barrier reservoir retains a supply of water for a hydroelectric plant, therefore the water level is under artificial control. The level depends on the inflow of water to the reservoir in relation to that taken out for the generation of electricity or released, unused, through the spillway. Fig. 3 shows the fluctuation of the water level at selected dates from July, 1947, until July, 1949. Inflow of water to the reservoir for this period is also plotted. The typical sequence of control for one year is as follows: drawdown is begun in late March or early April, continuing until a minimal volume of water remains

in the reservoir in May. This low water level is reached just prior to the spring runoff, which usually exceeds the average inflow and so refills the reservoir. Near-maximum volume is retained throughout the remainder of the year. After the spring runoff the inflow quickly assumes summer proportions, later declining to a winter minimum.

Exceptional conditions of spring inflow were encountered in 1948 and 1949. In 1948 the spring inflow was marked by a maximum considerably above the expected average maximum of 3000 cu. ft. per second (c.f.s.). More than

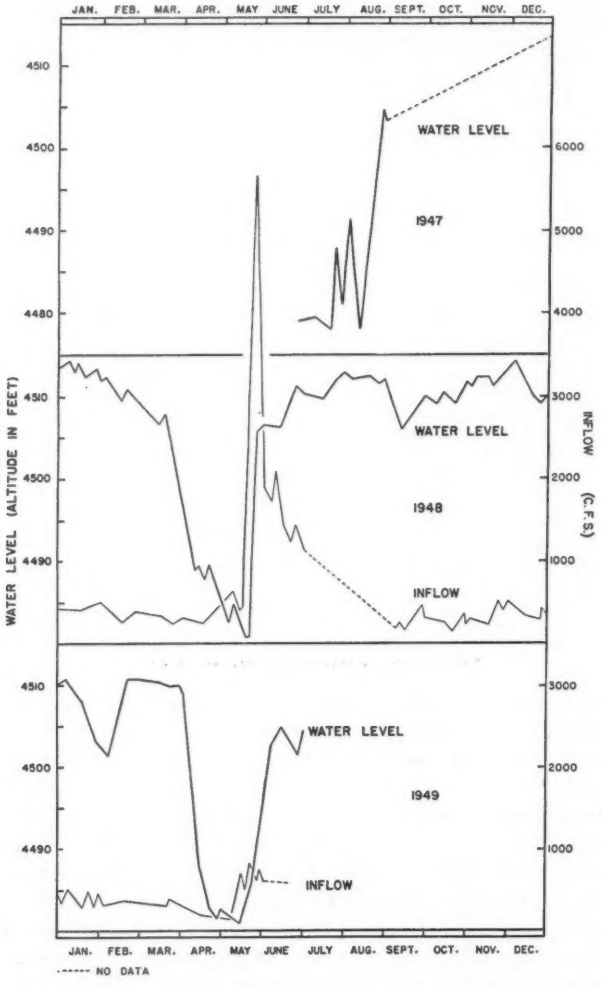

FIG. 3. Record of the water level fluctuation and the water inflow, Barrier reservoir, 1947–1949. (– – – – Indicates no data available.)

5000 c.f.s. was attained as the average for May 23, and it is believed that some hourly inflows averaged as high as 7000 c.f.s. The surge of water into the reservoir during this spring carried great quantities of sediment which were deposited throughout the basin.

Conditions were reversed in 1949 when there was no appreciable spring inflow, a result of light precipitation over the drainage area during the preceding winter. Consequently, the reservoir was filling very slowly at the termination of the investigation in June of that year. Large areas of the reservoir floor were left exposed for more than two months and sedimentation from the river was not noticeable.

The conditions encountered during these two spring seasons were favorable for limnological investigation since they showed the extremes of the range of conditions that can occur within this reservoir. The circumstances of the spring of 1949 were especially significant for they emphasized the yearly exposure of large stretches of littoral floor. Region I is most affected, for more than one third of its area is left dry at the time of maximum drawdown. Large, potentially productive areas are made barren, precluding the establishment of a stable biological environment. In addition to desiccation, erosion and scouring are pronounced over exposed areas as the water level rises and falls.

Replacement of Water Within the Reservoir

The volume of the Barrier reservoir at full supply level is approximately 1661 million cubic feet. The average inflow for 297 days during 1948 (inflow figures for July, August, and part of September not being recorded) was 601.3 c.f.s. This amounts to an annual inflow of approximately 19,000 million cubic feet or 11.4 times the total volume of the Barrier reservoir. In other words, the water within the reservoir is replaced 11.4 times per year, or almost once a month.

Data concerning the replacement of water within lakes are scarce. Strøm (11, 12, 13) records the "theoretical renewal" of water within several Norwegian lakes (Table III). Strøm's figures are based upon the mean annual amount of precipitation within the catchment (drainage) area and therefore upon the assumed amount draining into the lakes, evaporation being taken into account. These lakes occupy basins of divers origins; together they indicate that the rate of water renewal or replacement generally is much slower than that found for the Barrier reservoir. Only Øvre Sjodalsvatn compares with Barrier. Øvre Sjodalsvatn is a shallow lake lying in the Sjoa River valley; Barrier occupies a selected region in the Kananaskis River valley. A morphometric comparison of the two bodies of water is shown in Table IV.

As to the Barrier reservoir, the inflow to Øvre Sjodalsvatn varies with the season, being greatest in spring and minimal during late winter. Strøm's examination of Øvre Sjodalsvatn was brief, "conditions there being much too river-like for any conclusions to be drawn" (11). Recognition of the importance of water replacement in marking the character of a body of water is

TABLE III

COMPARISON OF RATE OF WATER RENEWAL (REPLACEMENT) BETWEEN THE BARRIER RESERVOIR AND VARIOUS NORWEGIAN LAKES

(Data from Strøm, 11, 12, 13)

Body of water	Mean depth, m.	Period of "theoretical renewal" years	Type of basin
Barrier reservoir	14.4	0.09	Dammed river valley
Øvre Sjodalsvatn	8	0.1	River valley
Lemonsjøen	15	7.0	Impounded by bottom moraine
Festhelsvatn	20	1.3	Cirque
Åvatn	27	1.6	Cirque
Bessvatn	29	8.5	Multiple cirque
Reinevatn	42	2.8	Cirque
Gjende	70	3.0	Fjord lake
Studalsvatn	73	1.1	Cirque
Solbjornvatn	75	10.8	Multiple cirque
Eikesdalsvatn	83	1.5	Glacial valley
Tennesvatn	92	5.1	Cirque
Mean period of renewal for Norwegian lakes		3.9	
Mean period excluding Sjodalsvatn		4.27	

TABLE IV

COMPARISON OF THE BARRIER RESERVOIR AND ØVRE SJODALSVATN

	Altitude, m.	Surface area, sq.km.	Drainage area, sq.km.	Maximum depth, m.	Mean depth, m.	Length, km.	Breadth, km.	Shoreline length, km.	Shore dev't.
Barrier	1375	3.09	859	39	15.4	3.65	0.64	16.94	2.25
Øvre Sjodalsvatn	950	4.94	475	23	8	6.3	1.2	20	2.54

evident in this remark. The importance of replacement will be demonstrated by the data from the Barrier reservoir, although there conditions must be less extreme than those suggested for Øvre Sjodalsvatn.

Sedimentation

The characteristic soil of the unflooded Kananaskis River valley at the reservoir site was a podsol, interrupted and overlaid by alluvium along flat stretches adjacent to the river. Gravel beds and bars, barren of vegetation, occurred along the river. Rock outcrops from the bases of the mountains around the reservoir site also intruded into the floodable area. With the impoundment of water the leaf litter of the vegetation-producing areas was

spread more or less evenly across the floor of the reservoir. Some concentration of this debris along southern and eastern shores, especially in shoreline indentations, was affected by the action of the current and the prevailing westerly winds. Very little sediment was deposited during the first year of the reservoir (1947) so the bottom was typically a mat of leaf litter and woody debris.

The above-average inflow of water during May, 1948, radically altered the character of the bottom. A heavy silt load was carried into the reservoir by the Kananaskis river. In region I coarser particles were deposited to form a thick (up to 50 cm.) layer of alluvium in the southern half of the region. Finer particles were carried further and were deposited as a soft, gray clay quite evenly throughout region II and the remainder of region I. The leaf litter was completely masked. Fig. 4 shows the pattern of alluvial deposits as they appeared in region I in June, 1949.

During the spring of 1949, when an extremely light runoff occurred, a different mode of sediment deposition took place. Little deposition from the river occurred. Instead, the erosion of material from the shores of the reservoir was accelerated by the slow and irregular ascent of the water level. Yellow clay sediments from the shores provided the main deposits of 1949,

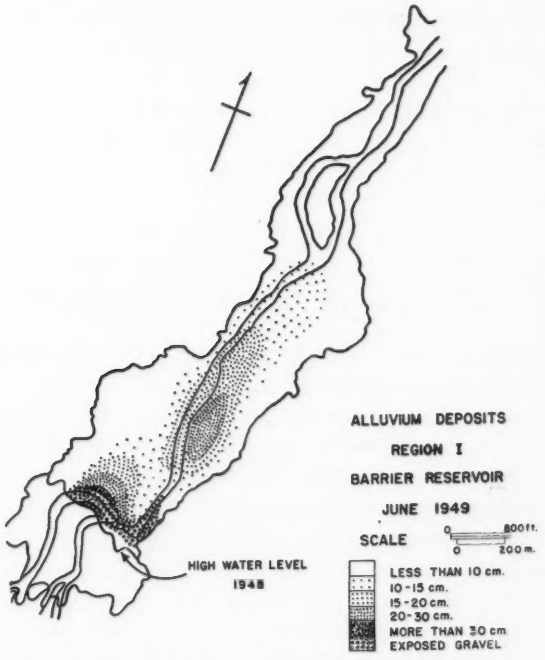

FIG. 4. Alluvium deposits in region I of the Barrier reservoir, as observed during June, 1949.

spreading in a layer 1 to 2 mm. thick over the floor of the reservoir. The color distinction of yellow for shore-formed sediments and gray for river sediments is quite clear. The validity of the distinction was confirmed by examining the boundaries of the two types of sediments in the exposed areas at the southern end of region I (June, 1949).

Local conditions around the margin of the reservoir resulted in varying contributions to the amount of sediment originating from the shores. For example, hydraulic washing of the south shore of region II beside the dam released great quantities of clay. This was done in an attempt to plug a hidden leak in the dam. The result was a hard, yellow clay bottom, several centimeters thick, for the eastern end of the reservoir south of the dam. A similar example is provided by the borrow pit used to supply material for the construction of the dam. The pit lies at the northeast corner of region II and remains as a bare clay plot, several acres in extent. Surface erosion by runoff water and erosion by the reservoir at the lip of the pit has resulted in an above average deposition of clay offshore from that locality.

About 75% of the shore length is made up of clay; gravel forms about 20% of the length, and rock outcrop the remaining five per cent.

Bottom Deposits in 1949

Stratification of bottom deposits from surface down may be described as follows:

Layer A.—A layer of indeterminable thickness, probably never exceeding 1 mm. Light brown and finely flocculent. Probably chiefly organic in nature, e.g., plankton remains, etc. Mineral sediments will settle through this layer to rest upon the next.

Layer I.—1–2 mm. yellow clay. Fairly compact; may be finely striated. This is sediment originating from shore materials, chiefly deposited during the spring of 1949. Thicker strata are localized in eastern region II as described above.

Layer II—3–30 mm. soft, gray clay. Light striae occasionally appearing in the top 1–2 mm. suggest some intermixture with Layer I. Layer II represents the sediments deposited by the Kananaskis river during the spring of 1948. The layer becomes thicker as the point of entrance of the river into the reservoir is approached (Fig. 4). In southern region I this layer is represented by a coarse, brown alluvium which attains a thickness of more than 50 cm. near the river entrance. At the extreme eastern end of the reservoir layer II is quite solid, owing to intermixture with the sediments originating from the shores.

Layer III.—A thin, black ooze layer covering the leaf litter of the original floor. The mineral constituents of this layer are assumed to come from layer II, while the color is the result of the processes of decay within the leaf litter.

Layer IV.—Leaf litter and debris, now blackened in decomposition. Because of its compressibility and coarse, variable nature it is difficult to measure its thickness accurately. Deposits thicker than 10 cm. were often encountered but the probable average thickness is 2–4 cm.

Basal layer—the original valley floor of podsol, alluvium, etc.

The layers described above were observed in 1949. Since then additional annual increments of sediment have been deposited. They will vary in composition and thickness according to the conditions under which they were deposited. They, and future deposits, will provide clues to the conditions that have governed the metabolism of the lake, when they are examined by investigators in the future.

Physicochemical Conditions

Table V summarizes the physical and chemical conditions found in the Barrier reservoir during the several periods of investigation. It is to be noted that the depth of the bottom varies at the different times of sampling according to the variation in the water level in the reservoir.

The greatest temperature difference between surface and bottom waters occurred July 10, 1947, when a difference of 6° C. was recorded over a depth of 16.5 m. There was never any oxygen stagnation, although on July 26, 1947, the bottom oxygen decreased to about 53% of saturation. Over the entire period of the investigation the oxygen content of the surface water averaged 90.1% of saturation. The oxygen content of the bottom water, including the reading for July 26, 1947, had a mean value of 61.3% of saturation. The water remained alkaline; pH 7.4 (July 26, 1947) was the least alkaline value recorded.

The uniformity of the various values can be attributed to the rapid replacement and consequent circulation of water within the reservoir. The incipient stagnation indicated by a decrease in oxygen saturation and an increase in hydrogen ion concentration discovered on July 26, 1947, occurred during a period when no water was released from the reservoir, i.e., when circulation was at a minimum. During the week following July 26 a large quantity of water was released from the reservoir. More was released during August and early in autumn the power plant initiated operations. Subsequently no sign of stagnation appeared.

Plankton

The plankton of the Barrier reservoir is scanty in number and variety. The establishment of a plankton population was rapid during the summer of 1947 but since that season the increase in numbers and species has been slow. The Entomostraca are represented chiefly by copepods (*Cyclops*, *Diaptomus*) and less frequently by cladocerans (*Bosmina*, *Chydorus*). Rotifers, chiefly *Anuraea*, are common. *Dinobryon* and *Ceratium* are the major representatives of the Protozoa. Diatoms are the most abundant algae, *Asterionella*

TABLE V

PHYSICOCHEMICAL CONDITIONS OBSERVED WITHIN THE BARRIER RESERVOIR, 1947–49

	1947							1948		1949	
	June 2	June 23	July 10	July 26	Aug. 13	Aug. 29	Sept. 10	Jan. 14	June 30	Jan. 11*	June 6*
Temperature °C.											
Air	10	17.2	21.5	12.5	12.2	12.0	—	—	—	—	19.5
Surface	8.5	11.8	13.1	13.6	12.2	11.3	10.7	0.35	12.3	—	11.7
5 m.	8.0	8.2	9.5	11.5	11.2	11.2	—	—	—	—	—
10 m.	6.2	7.5	8.0	9.1	10.4	11.0	10.3	—	8.3	—	7.1
15 m.	—	—	—	—	—	9.3	—	2.3	6.8	—	6.0
Bottom	5.9	6.8	7.1	8.5	9.3	9.0	9.4	3.2	5.9	—	—
(Depth in m.)	(16.5)	(16.0)	(16.5)	(19)	(17.5)	(24)	(24)	(28)	(27)		
Dissolved O_2 cc. per liter											
Surface	6.0	5.5	5.6	5.7	5.9	6.4	5.7	8.1	6.1	—	5.4
5 m.	5.9	5.5	5.6	5.7	5.3	5.9	—	—	—	—	—
10 m.	5.8	5.6	5.5	5.0	5.7	6.3	5.7	—	—	—	—
Bottom	5.6	5.6	4.5	3.7	5.9	5.0	5.7	5.5	5.4	4.0	5.3
(Depth in m.)	(16.5)	(16.0)	(16.5)	(19)	(17.5)	(24)	(24)	(28)	(27)	(25)	(26)
pH											
Surface	8.0	7.8	8.3	8.0	8.0	8.2	8.0	—	8.3	8.3	—
Bottom	7.8	7.7	8.0	7.4	7.8	7.8	8.0	—	8.2	8.3	—
Secchi's disk											
Meters	1.3	3.2	3.4	1.0	3.2	2.9	2.6	—	2.0	—	2.3

* *Incomplete results due to inaccurate recording by reversing thermometer.*

TABLE VI

COMPARISON OF AVERAGE STANDING CROPS OF PLANKTON OF SEVERAL BODIES OF WATER, INCLUDING THE BARRIER RESERVOIR

Body of water	Plankton, dry wt. kgm./ha.	% Organic matter
Barrier reservoir		
Aug., Sept., 1947	10.4	55
Jan., June, 1948	11.0	75
Jan., June, 1949	10.7	74
Ghost river reservoir	10.0	60
Upper Kananaskis lake	17.9	81
Lower Kananaskis lake	32.6	82
Bow lake*	11.9	67
Minnewanka*	32.0	80
Paul lake*	50.2	84

* *Data from Rawson* (7).

occurring most frequently. The standing crop of plankton of Barrier is compared with averages of standing crop determinations in other bodies of water in Table VI.

The figures quoted for the Barrier reservoir are somewhat misleading owing to a high degree of contamination of the samples by silt (1947) and by wood fibers (all periods). The result is an exaggeration of the weight. Use of ashing techniques circumvented the difficulties introduced by silt contamination but the error due to organic contamination is difficult to avoid. Rawson (8) estimated that the actual weight of plankton in Barrier during 1947 was only about one half as great as that of the Ghost River reservoir. The amount of contamination was reduced somewhat during 1948 and 1949, but the actual weight of the plankton probably was not as great as that of the Ghost River reservoir in 1949. Table VI shows the plankton crop to be less than that of any of the other bodies of water considered, all of which are oligotrophic. The Barrier crop is but one fifth the weight of that occurring in Paul lake, which, while oligotrophic, supports a weight of plankton extending into the eutrophic range (7).

Bottom Fauna

The bottom fauna of the Barrier reservoir consists of larvae of Chironomidae, Oligochaeta, *Gammarus*, and larvae of several dipterous families other than Chironomidae. In addition, there are larval Ephemeroptera and Plecoptera washed into the reservoir from the Kananaskis River. Table VII compares the abundance of the Chironomidae with the other benthic forms present. The abundance of the chironomids makes them the chief subject of the discussion to follow.

TABLE VII

NUMBERS OF CHIRONOMIDAE COMPARED TO NUMBERS OF OTHER BENTHIC ORGANISMS IN THE BARRIER RESERVOIR, 1947–49

Year	Sampling period	Chironomidae		All others		Totals		% Chironomidae	
		No./sq.m.	Wt./sq.m., gm.	No./sq.m.	Wt./sq.m., gm.	No./sq.m.	Wt./sq.m., gm.	No./sq.m.	Wt./sq.m., gm.
1947	June	16	.015	46	.669	62	.684	25.8	2.2
	July	123	.177	128	.475	251	.652	49.0	27.2
	Aug.	335	.285	3	.004	338	.291	99.2	94.2
	Sept.	390	.949	26	.024	416	.973	93.6	97.5
1948	Jan.	1270	6.641	6	2.769	1276	9.410	99.6	70.5
	June	134	.802	15	.114	149	.916	89.9	87.5
1949	Jan.	776	1.035	4	.009	780	1.044	99.6	99.2
	June	381	.841	17	.062	398	.903	95.7	93.3
Totals		3425	10.747	245	4.126	3670	14.873	88.0	72.4
			Total, excluding June, July, 1947					98.0	79.4

Identification of chironomid specimens was made by the author, using keys to larvae constructed by O. A. Johannsen (1, 2). The names used in this paper are according to these references. Identification of specimens was limited to larval forms, not taken beyond genus, for chironomids of this area are poorly known.

In order to simplify the discussion of the chironomids, generic names will be used to represent larger groups. Members of the tribe Chironomariae and Tanytarsariae (subfamily Chironominae) will be referred to as Chironomus and Tanytarsus, respectively; exception shall be made for *Pentapedilum* (Chironomariae) which shall be referred to in its generic sense. Tanypus will indicate the subfamily Tanypodinae; Orthocladius and Diamesa will represent the Orthocladiinae and Diamesinae.

Because the investigation of the Barrier reservoir was undertaken at specific periods during the course of two years and because the pattern of bottom population development becomes apparent as the data from each period are examined in order, the description of the population changes will be presented in chronological order. Fig. 5 summarizes these data.

Development of the Benthic Population

June, 1947

This was the second month of flooding and the first period of sampling within the reservoir. The water level averaged about 4880 ft., or 35 ft. (10.7 m.) below full supply level. The floor of the reservoir was covered with leaf litter.

The bottom fauna were few in number. They comprised *Pentapedilum*, oligochaetes, and various survivors of the now-covered river, such as mayfly

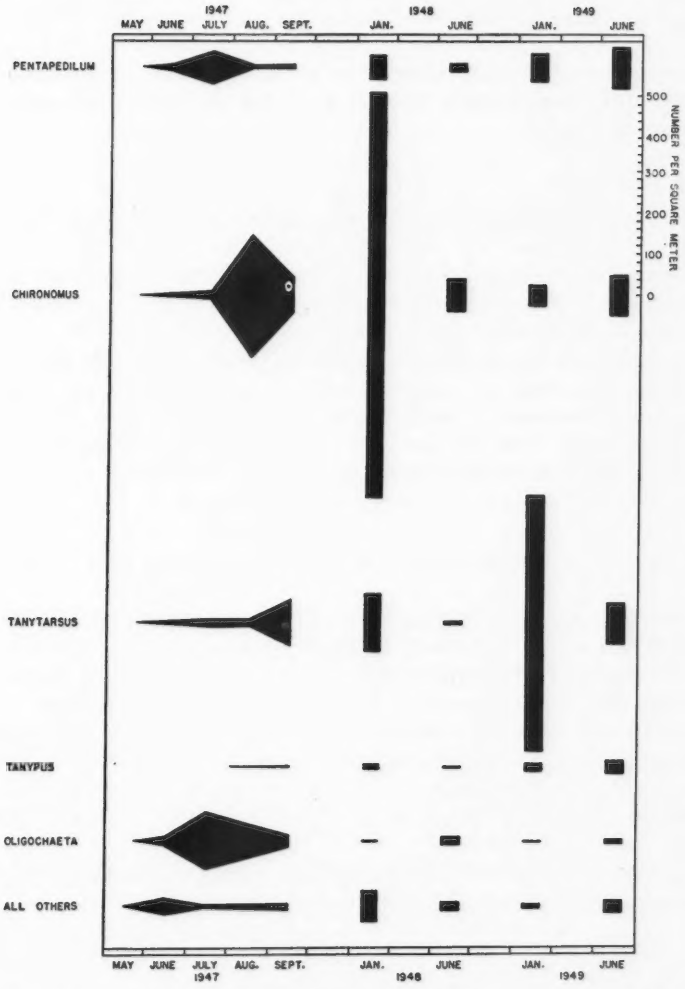

FIG. 5. Development of the bottom fauna of the Barrier reservoir from May, 1947, until June, 1949.

and stonefly nymphs, planarians, *Gammarus*, and tipulid larvae. Tanytarsus appeared infrequently in the samples collected. Chironomus was not encountered.

July, 1947

The water level averaged about 4481 ft., or 34 ft. (10.4 m.) below full supply level.

Chironomus appeared in bottom samples for the first time, becoming second in importance to *Pentapedilum* among the Chironomidae. Tanytarsus

appeared in greater numbers than during the previous month. Oligochaetes were the most abundant benthic organism, however, being twice as numerous as *Pentapedilum*. The extraneous mayflies and stoneflies appeared much less frequently than during June. During July was noted the first appearance of chironomid pupae.

August, 1947

The water level averaged about 4495 ft., or 20 ft. (6.1 m.) below full supply level.

The paucity of the samples collected during August (five only) detracts from their value as data for generalization. However, certain features of the population trend are observable.

The numbers of individuals of Chironomus increased greatly; the numbers of *Pentapedilum* decreased greatly, probably as a result of first generation emergence. Tanytarsus showed little change in numbers from July. Orthocladius was found for the first time. Neither river forms nor oligochaetes were collected, although both groups undoubtedly were present.

September, 1947

The water level averaged about 4507 ft., or 8 ft. (2.4 m.) below full supply level.

Chironomus was the dominant benthic organism, *Pentapedilum* being restricted still more than during August. Tanytarsus showed an increase in number and exhibited the phenomenon of localization noted by Muttkowski (4) in lake Mendota, Wisconsin. For example, one dredging sample (SI D9) contained 136 Tanytarsus individuals, the equivalent of 2052 individuals per square meter. This is in sharp contrast to the average of 34 Tanytarsus per square meter for the remainder of the reservoir and is widely divergent from the results of other dredgings taken the same day, at the same depth, at different locations in the lake.

Oligochaetes were less abundant than during July. Orthocladius increased in number. Tanypus and Diamesa appeared for the first time.

January, 1948

The floor of the reservoir was still covered with leaf litter, i.e., no appreciable sediment deposition had yet occurred. The water level averaged about 4513 ft., only 2 ft. less than full supply level.

The benthic population of the reservoir during the winter of 1947-48 was notable for the large number of individuals composing it. The number of organisms present per square meter of bottom area was 1276, more than one and one-half times the number present at the next greatest period of abundance, January, 1949.

Chironomus comprised 77% of the 1948 winter population. The chief species was a large, red, bathyphilous form. Tanytarsus had increased to comprise 11% of the total numbers. *Pentapedilum* was present in numbers

equal to its midsummer maximum of July, 1947. Orthocladius increased in number; Tanypus, Diamesa, and oligochaetes were present but not abundant. However, several large lumbriculid oligochaetes did contribute significantly to the weight of the samples. This was the only time at which such oligochaetes appeared in the samples.

June, 1948

The exceptional spring runoff of 1948 had materially altered the nature of the bottom of the reservoir. The leaf litter of 1947 was now covered by soft clay and alluvial deposits of varying thickness. The water level averaged 4509 ft., or 6 ft. (1.8 m.) below full supply level.

The results of the June, 1948, examination were in striking contrast to those of January, 1948, i.e., a minimum of abundance was encountered. The minimum was interpreted as the result of the emergence of overwintering larvae, with the establishment of a new generation not yet completed. Furthermore, it is possible that the heavy sediment deposition by the Kananaskis river had a detrimental effect on the re-establishment of a chironomid population by causing a heavy mortality either among potential adults before emergence or among newly laid eggs or young larvae.

The number of organisms averaged only 149 per square meter, less than for any period of sampling except June, 1947. The average live weight of organisms per square meter was 0.916 gm., which was greater than the average for June, July, or August, 1947, or for June, 1949. The apparent discrepancy between relative numbers and weights can be accounted for by the fact that nearly 55% of the organisms collected in June, 1948, were the large bathyphilous Chironomus. During 1947 the bathyphilous form had not become dominant, and by 1949 it had been supplanted by the smaller Tanytarsus and other Chironomus forms.

Tanypus, Orthocladius, and Diamesa were present in unexceptional numbers The oligochaetes showed a slight increase in numbers.

January, 1949

The surface layers of the bottom deposits was the soft, gray clay brought in by the Kananaskis river during the spring of 1948. The water level averaged 4510 ft. or 5 ft. (1.5 m.) below full supply level.

The second winter of existence for the Barrier reservoir was marked by another period of increased abundance of chironomid larvae and a decrease in numbers of oligochaetes. On the basis of total numbers and total weight the 1949 maximum did not approach that of 1948. Of greater significance than the numerical totals, however, was the fact that the composition of the benthic population had greatly altered. Tanytarsus rose to dominance, while Chironomus, especially the bathyphilous form, decreased greatly in number. At this time Tanytarsus comprised 79% of the population. Chironomus made up only 7%. *Pentapedilum* and Tanypus achieved greater maxima than they had in the past. Diamesa remained limited in number, while Orthocladius and the oligochaetes were very rare.

June, 1949

The bottom deposits were altered by a layer of yellow clay from the shores, laid over the other sediments. The water level averaged 4503 ft., or 12 ft. (3.7 m.) below full supply level.

The spring of 1949 was advanced and the runoff through the Kananaskis river was considerably less than the expected average. Consequently, the re-establishment of the chironomid population within the reservoir was well advanced by the time of examination. Instead of this period being the time of the spring minimum, it appears that the first summer maximum was approaching. All the faunal groups of the benthos showed an increase over the numbers present during January, 1949, with two exceptions: Tanytarsus which appeared minimal and was represented by many pupae, and Diamesa, which showed no change from the numbers present during the winter. The bathyphilous Chironomus was still fairly common, but the proportion of smaller Chironomus forms was increasing.

Examination of the broad littoral shelf along the north shore of region II, exposed by the low water level, revealed the husks of numerous amphipods. The remains were not identified, but their size suggested *Gammarus*. This was the first trace of *Gammarus* found within the reservoir since June, 1947. *Gammarus* and *Hyalella* were planted in the reservoir in September, 1947 (8). *Gammarus* are plentiful in shallow pools along the Kananaskis river near its entrance into the reservoir. It is evident that the amphipods are spreading into the reservoir, but their advance may be delayed by the lack of a well-developed littoral zone.

Control of the Benthic Succession

Physical, chemical, and biological conditions within a lake are interrelated and may be considered as being governed, in large part, by edaphic, climatic, and morphological factors in action at the location of the lake and over its drainage area. Rawson (5, 6, 7) has discussed the contributions of these factors. In the Barrier reservoir these factors act strongly toward oligotrophy.

In the Barrier reservoir artificial interference has modified the effect of water replacement to an extent that markedly affects the physical and chemical conditions within the reservoir, which in turn influence the biological processes. The interference acts in such a manner as to increase the tendency toward oligotrophy. The total effect of this may now be considered.

The first benthic organisms found within the reservoir were the miscellaneous forms surviving from the river. By the end of the first summer these forms had dwindled to insignificance, no doubt as a result of their inability to survive in a lentic environment. Occasional specimens of river fauna were still collected from the southern part of region I in June, 1949. These had been washed into the reservoir from the Kananaskis river. Mayfly, stonefly, and caddis larvae were found in that order of abundance. Rarely did these organisms penetrate more than 100 to 200 yd. into the reservoir.

Pentapedilum was first among the chironomids to become dominant. This dominance, clearly demonstrated during July, 1947, was followed by decline in the two succeeding months. The decline probably indicated the emergence of adults. *Pentapedilum* remained a significant member of the population, but fell from dominance as other species achieved numerical superiority.

Chironomus, chiefly a bathyphilous form, was the dominant benthic organism by September, 1947. The dominance of this group was maintained until the spring of 1948.

Tanytarsus was prominent by September, 1947. Overshadowed by Chironomus during the following winter, this group probably became dominant during the summer of 1948. By January, 1949, it was firmly established as the dominant benthic organism. During June, 1949, Tanytarsus, Chironomus, and *Pentapedilum* approximated each other in numbers. The presence of abundant Tanytarsus pupae suggests that the spring minimum for Tanytarsus occurred later than the minima for the other two groups, which resulted in a temporary suppression from dominance for Tanytarsus.

These data, so generally compared, indicate that within two years three forms have succeeded each other as benthic dominants, viz.:

Pentapedilum ——→ Chironomus ——→ Tanytarsus.

This succession within the Barrier reservoir can be readily explained. *Pentapedilum* was briefly dominant because it was the first chironomid established in the reservoir. Chironomus, a eutrophy-typical form, took advantage of the nearly optimum eutrophic conditions presented by the leaf litter to become dominant. The decomposing leaf litter was rich in the organic food material that Chironomus accepts readily (3, 9, 10). As a result of the heavy spring runoff of 1948, the leaf litter was completely buried, the eutrophic conditions disappeared and oligotrophy-typical Tanytarsus became dominant, to reflect the more severe environment.

The supplementary chironomid forms found in the Barrier reservoir rarely achieve dominance among the bottom fauna of any body of water. Orthocladius and Diamesa are common phytophagous larvae. Tanypus, a predaceous larva, usually depends upon the development of other chironomids for its own establishment; Tanypus showed a gradual increase in numbers from 1947 to 1949. Similarly larvae of *Cryptochironomus*, predaceous members of the Chironomus group, were becoming more numerous as the benthic population developed.

These data are taken to mean that the succession of benthic organisms have indicated a rapid trophic change along the floor of the Barrier reservoir. An early, eutrophy-like benthic environment has been supplanted by an oligotrophic condition more in keeping with the edaphic, climatic, and morphological factors affecting this mountain lake.

Vertical Distribution of the Bottom Fauna

The fluctuation of the water level of the reservoir results in a frequent reassignment of the depth zones. However, the fluctuation is slow enough, except during early spring, to allow for the development of concentration

zones for the organisms which show a preference for inhabiting the bottom at certain depths. In the Barrier reservoir Chironomus and Tanytarsus appeared to prefer certain depths, as shown by the concentration zones of these organisms shifting location as the changing water level varied the depth of water throughout the lake. *Pentapedilum* appeared to be less influenced by variations in depth.

Changes in the concentration zones of Chironomus, Tanytarsus, and *Pentapedilum* are illustrated in Fig. 6. The vertical distribution of Chironomus shows greater variation than that of any other group. Chironomus first

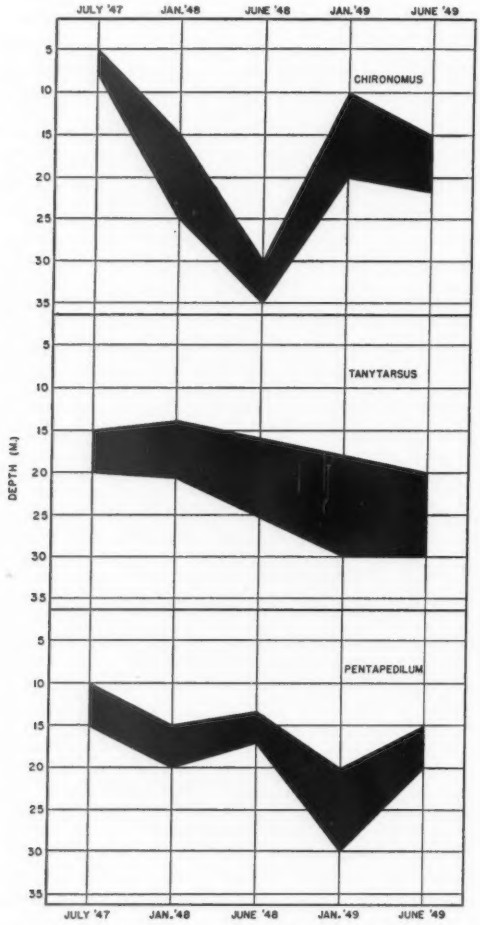

FIG. 6. Vertical distribution of the major groups of Chironomidae present in the Barrier reservoir from July, 1947, until June, 1949. The depths shown are actual depths, not potential depths plotted from full supply level.

appeared in shallow water, then its zone of concentration moved into the profundal area. The downward displacement took place as the bathyphilous members of the group became increasingly numerous. Then, following the environmental changes of the spring of 1948 and the suppression of the bathyphilous Chironomus forms, the zone of concentration ascended rapidly. At the end of the investigation (June, 1949) the vertical distribution showed a zone of concentration at 15–20 m. This is 10 m. above the deepest zone established by Chironomus.

The vertical distribution of Tanytarsus remained nearly the same throughout the investigation. This organism was most abundant between 15–25 m. The slight tendency of the zone to descend may have been exaggerated in Fig. 6 by the data of the June, 1939, examination. At that time Tanytarsus was minimal in number. The specimens collected were chiefly from the deeper levels, those of shallower water already having emerged as adults.

The vertical distribution of *Pentapedilum* suggests a winter descent and a summer ascent when plotted against actual depths as in Fig. 6. That this change is only apparent can be shown by plotting the distribution of *Pentapedilum* against depths at full supply level. This can be done by adding two meters to the depth of the concentration zone plotted for June, 1948, and four meters to the zone plotted for June, 1949. The trend of the vertical distribution is thereby straightened. In other words, had the lake level remained constant, the depth distribution of *Pentapedilum* also would have been constant. No change in the positions of the zones of concentration of Chironomus or Tanytarsus occurs when their vertical distributions are plotted against full supply level, i.e., their zones of concentration are correctly represented in Fig. 6.

Standing Crop of Bottom Fauna

In Table VIII the standing crop of bottom fauna in the Barrier reservoir at specific periods is compared with the crops found in several other bodies of water.

Group I includes the values obtained for Barrier.

Group II includes several bodies of water found along the Bow and Kananaskis rivers, in the same general region as the Barrier reservoir. Lower Kananaskis lake is one of two large lakes at the head of the Kananaskis river. The Kananaskis reservoir is at the confluence of the Bow and Kananaskis rivers; it is not intended for water storage, so shows few lakelike characteristics. The bottom is sandy and gravelly like that of a river, and the circulation of water is very rapid. Oligochaetes are by far the most abundant benthic organism. The Horseshoe reservoir, situated immediately downstream from the Kananaskis dam, is similarly riverlike. The Ghost river reservoir was established at the confluence of the Bow and Ghost rivers in 1930. It forms a lake eight miles in length. Lac des Arcs and Gap lake are small natural bodies of water connected to the Bow river several miles upstream from the Kananaskis reservoir. They are very shallow and support luxurious aquatic vegetation.

TABLE VIII

COMPARISON OF THE STANDING CROPS OF BOTTOM FAUNA OF THE BARRIER RESERVOIR WITH THOSE OF SEVERAL OTHER BODIES OF WATER

Group I Body of water	Barrier Sept. '47	Barrier Jan. '48	Barrier June '48	Barrier Jan. '49	Barrier June '49	—
Bottom fauna, dry wt. kgm./ha.	1.94	18.82	1.83	2.09	1.81	—
Group II **Body of water**	**Lower Kananaskis lake**	**Kananaskis reservoir**	**Horseshoe reservoir**	**Ghost river reservoir**	**Lac des Arcs**	**Gap lake**
Bottom fauna, dry wt. kgm./ha.	7.8	11.9	4.2	4.5	10.1	53.9
Group III* **Body of water**	**Paul lake**	**Minnewanka**	**Maligne**	**Bow lake**	**Okanagan**	**Waterton**
Bottom fauna, dry wt. kgm./ha.	36.4	4.5	3.3	2.3	2.0	1.7

**Data from Rawson* (7).

Group III includes a series of oligotrophic lakes in the Rocky Mountains, examined by Rawson (7).

It is evident that the benthic productivity of the Barrier reservoir is small. Only during the winter of 1947–48 did the standing crop of bottom fauna approach the eutrophic range. Of the lakes listed in Table VIII, Waterton alone presents a poorer crop. According to Rawson, Waterton lake is almost "harmonic" for factors resulting in low productivity, i.e., the effects of edaphic factors, the morphology of the basin and, to a lesser extent in this case, the climate, all favor low productivity.

The Kananaskis and Horseshoe reservoirs present constant near-optimum river conditions, whereas the Barrier reservoir is essentially a lake influenced by unfavorable conditions. Consequently, the qualitatively poor bottom fauna of the former two reservoirs can maintain a heavier crop than the fauna of the Barrier reservoir, which is subject to sudden environmental changes.

The changes of environmental condition within the Barrier reservoir, illustrated most clearly in the spring of the year, are probably the most important limiting factors to productivity. The youthfulness of the reservoir has also limited the crop; qualitative and quantitative improvement may be expected in the future. *Gammarus* is appearing and should assume greater importance. Mollusks, abundant in Lower Kananaskis lake upstream from the Barrier reservoir no doubt will appear. Many changes are to be expected within the limits imposed by the physical conditions affecting the reservoir.

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References

1. JOHANNSEN, O. A. Aquatic Diptera. Part III. Chironomidae: Subfamilies Tanypodinae, Diamesinae, and Orthocladiinae. Cornell Univ. Agr. Expt. Sta. Mem. No. 205. 1937.
2. JOHANNSEN, O. A. Aquatic Diptera. Part IV. Chironomidae: Subfamily Chironominae. Cornell Univ. Agr. Expt. Sta. Mem. No. 210. 1937.
3. LEATHERS, A. L. Ecological study of aquatic midges and some related insects with special reference to feeding habits. Bull. U.S. Bur. Fisheries, 38. 1922
4. MUTTKOWSKI, R. A. The fauna of lake Mendota. Trans. Wisconsin Acad. Sci. 11 : 374–454. 1918.
5. RAWSON, D. S. The bottom fauna of lake Simcoe and its role in the ecology of the lake. Pub. Ont. Fisheries Lab. No. 40. 1930.
6. RAWSON, D. S. Some physical and chemical factors in the metabolism of lakes. Problems of lake biology. Am. Assoc. Advancement Sci. Pub. No. 10 : 9–26. 1939.
7. RAWSON, D. S. A comparison of some large alpine lakes in western Canada. Ecology, 23 : 143–161. 1942.
8. RAWSON, D. S. Biological investigations on the Bow and Kananaskis rivers. Unpublished report to Calgary Power Co. and Calgary Fish and Game Association. 1948.
9. REMPEL, J. G. The life-history and morphology of *Chironomus hyperboreus*. J. Biol. Board Can. 2(2) : 209–221. 1936.
10. SADLER, W. O. Biology of the midge *Chironomus tentans* Fabricus, and methods for its propagation. Cornell Univ. Agr. Expt. Sta. Mem. No. 173. 1935.
11. STRØM, K. M. Bessvatn. and other lakes of eastern Jotunheim. Skrifter Norske Videnskaps-Akad. Oslo. I. Mat.-Naturv. Klasse. 4 : 1–32. 1935.
12. STRØM, K. M. Eikesdalsvatn. A limnological reconnaissance. Skrifter Norske Videnskaps-Akad. Oslo I. Mat.-Naturv. Klasse. 8 : 1–11. 1937.
13. STRØM, K. M. Moskenesøy. A study in high latitude cirque lakes. Skrifter Norske Videnskaps-Akad. Oslo. I. Mat.-Naturv. Klasse. 1 : 1–32. 1938.
14. WELCH, P. S. Limnological methods. The Blakiston Company, Philadelphia. 1948.

THE ENTOMOSTRACA OF SOUTHERN SASKATCHEWAN[1]

By J. E. Moore[2]

Abstract

Forty-nine species of Entomostraca were found in 48 lakes situated in the southern half of Saskatchewan. These lakes form a series ranging from fresh to extremely saline waters. The salinity in most cases was less than 10,000 p.p.m. The occurrence of Entomostraca is correlated with the degree of salinity. Total concentration is considered to be of prime importance in determining presence or absence of species. There were pronounced differences in the total salt content of waters inhabited by the various species. Cladocera were more markedly restricted by high salinity than were Copepoda. Littoral species were apparently more susceptible to increasing concentration than were those of limnetic habits. Typical entomostracan communities in these lakes are discussed. The communities in highly saline waters are quite comparable to those reported in similar habitats in other parts of the world. Marked alterations in the fauna of two lakes have resulted from salinity changes over a period of 20 years. Tentative upper limits of salinity tolerance for many of the species have been proposed. Salt concentrations of between 4000 and 16,000 p.p.m. appear to be upper limits for most freshwater types. The majority of species are considered to possess no special osmoregulatory mechanisms. Except for *Artemia salina* and *Moina hutchinsoni* the Entomostraca present in these lakes are typically freshwater species which in general have a wide geographic distribution. The fauna has undoubtedly been derived from many sources and in only a few instances are there indications of definite points of origin.

Introduction

The existence of saline lakes in the southern half of the Province of Saskatchewan has been known for many years but until recently little was learned about their ecology. In 1920, the Quill Lakes were studied by Huntsman (21) from the standpoint of fishery possibilities and, except for Bajkov's examination of these and one additional lake (4), no further scientific work was done until 1938. An extensive limnological survey of the whole area was begun by the writer in the summer of that year and continued during 1939, 1940, and 1941. This investigation has provided much information relating to the physical, chemical, and biological nature of the lakes. A detailed treatment of physical and chemical data and a general discussion of biological features have been presented by Rawson and Moore (38). The same paper also contains a description of the geological and physiographic features of the area under consideration. Certain special groups of organisms in these waters have been dealt with by Walker (42), Ide (25), and Kuehne (29). The present study which deals with the distribution of Anostraca, Cladocera, free-living Copepoda, and Ostracoda in 48 lakes, is a further contribution to our knowledge of the aquatic fauna of this region.

The flora and fauna of inland salt lakes have attracted the attention of many investigators and as a result a fairly extensive literature is now available. Information on the distribution of Entomostraca in saline lakes of North

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America comes largely from the works of Kemmerer, Bovard, and Boorman (27), Young (48), Hutchinson (23), and Willey (45). Several writers such as Florentin (15) and Schäfer (40) have indicated the maximum salinities for various species in European waters. Among the more detailed studies of saline waters in other parts of the world are those of Hutchinson, Pickford, and Schuurman in South Africa (24), Decksbach in Siberia (14), and Beadle in Algeria (6).

Methods and Materials

The following is a summary of the general features of the investigations on which this study is based. Surveys in 1938 and 1939 were of a preliminary nature designed to ascertain the physical, chemical, and biological conditions in as many lakes as possible. In 1940 the inquiry consisted of an intensive examination of eight lakes having salinities which were representative of the range of concentrations encountered in previous years. The study in 1941 consisted primarily of a detailed investigation of a single lake with brief visits to four other lakes for the gathering of supplementary information. In each year, field work was carried on from May until September.

Areas were determined chiefly from large scale maps of various sections of the Province. The levels of many of these waters have dropped considerably since the maps were prepared but the calculation of the resulting decrease in area was beyond the scope of our studies.

The depths of most lakes were determined by means of soundings. The number of soundings was usually sufficient to reveal maximum depths but only in a few instances provided enough data for calculating average depths.

A Negretti-Zambra reversing thermometer was used to record temperatures at different depths.

Surface and deep water samples were taken with a brass water bottle and analyses of oxygen and hydrogen ion concentration were made at the time of collection. Miller's method for determining the quantity of dissolved oxygen was used. The hydrogen ion concentration was determined colorimetrically using La Motte standards for phenol red and thymol blue indicators.

A measure of transparency was obtained by using Secchi's disk according to the standard procedure. The disk, 20 cm. in diameter, was made of white oilcloth attached to a wire frame. All readings were taken in the open water regions of the lake.

Water samples for mineral analyses were strained through linen cloth into either 1-gal. Winchesters or 8-oz. bottles, depending on the salt content of the water. Determinations of total residues and common inorganic constituents were made by Dr. W. E. Gordon at the Department of Chemistry, University of Saskatchewan, under the direction of Dr. T. T. Thorvaldson. The procedure in waters of low to moderate salinity followed that given in Standard Methods of Water Analysis (1) but for high concentrated waters special techniques were necessary, particularly in doing magnesium determinations.

Plankton samples were taken with a 20-liter trap and also by the use of nets. The net collections consisted of littoral tows, surface tows, and total vertical hauls from the open water. The first type was made with a Wisconsin net (26) while for surface tows and total vertical hauls a net patterned after the Wisconsin type but of larger size (mouth 25 cm. in diameter) was utilized. Nets were made of No. 20 silk (30,000 meshes per sq. in.). In taking the littoral and surface tows the net was drawn behind a boat which was rowed slowly for a period of three minutes. In making a total vertical haul the net was pulled up from the bottom of the lake at the rate of approximately one-half meter per second. Both surface and total vertical hauls were taken at the stations where physical and chemical observations were made. All samples were preserved in 5% formalin solution.

The plankton collections of 1938 consisted of surface tows and total vertical hauls from each of 24 lakes. Samples were taken from 16 of these lakes again in 1939 as well as from 23 additional lakes. A littoral tow was made in each lake visited in 1939 and, usually, surface and total vertical collections were taken also. One new lake was sampled by nets in 1940 but the bulk of the material for this year came from eight selected lakes in which both net and trap sampling methods were employed. Jackfish, Little Manitou, Murray, Redberry, and Turtle Lakes were visited three times, Last Mountain and Stoney Lakes were visited twice and Echo Lake on one occasion in 1940. Redberry Lake was studied throughout the summer of 1941 with samples of all types being taken at approximately weekly intervals. Small portions of the samples taken in 1938 were submitted to Dr. G. C. Carl, Provincial Museum, Victoria, who determined the Cladocera and Copepoda. The main samples were examined subsequently by the writer with the result that several rarely-taken species were added to the list. The collections of later years were identified by the writer.

Location and Morphometry of Lakes

The location of the 48 lakes from which plankton samples were obtained is indicated in the map, Fig. 1. All but three are found in that portion of Saskatchewan extending from latitude 50° north almost to 54°. Eighteen of them lie in the forest zone, 23 are in the parkland, and the remaining 7 in the prairie region. This survey included most of the permanent lakes, of any considerable size, in the prairie and parkland areas but only a small proportion of those in the forested region. Maps usually show several lakes in the southwest part of the Province but the majority of these have either dried up completely or become dry in the summer months. In Fig. 1 the lakes indicated with dotted outlines belong in this category.

Big Quill with an area of 162 sq. miles is the largest lake in southern Saskatchewan. Nine others with areas of 20 sq. miles or more may be considered large bodies of water. The remainder of those studied range from 17 to 0.12 sq. miles and most are less than 5 sq. miles in extent (Table I).

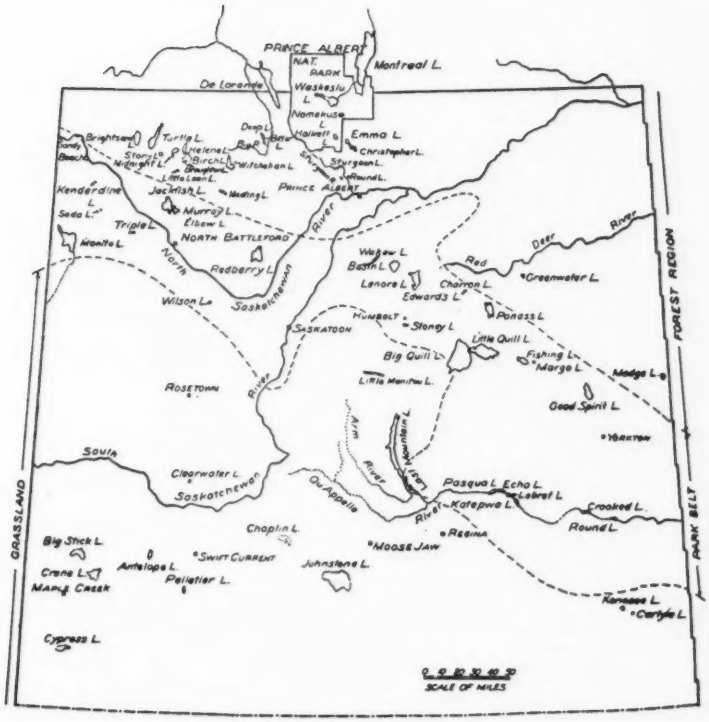


FIG. 1. Map showing location of lakes and also boundaries of vegetation zones in southern Saskatchewan.

Maximum depths in these waters vary from about 5 to 80 ft. The depths recorded in Table I are generally those of the deepest points at which observations were made and in most instances represent maxima. Figures for Atten's, Big Quill, Helene, Little Quill, Midnight, Pasqua, Sandy Beach, and Witchekan Lakes are estimated maximum depths. Nine of the lakes are at least 50 ft. deep but the majority are comparatively shallow (28 have maxima of 30 ft. or less).

Physical and Chemical Conditions

Temperature records (Table I) indicate that most of these waters become almost uniformly warm from surface to bottom during the summer months and that mean temperatures often reach 17 to 20° C. Such conditions may be ascribed mainly to insolation, frequent wind action, and the shallowness of most lakes. Bitter, Deep, Elbow, Manito, and Redberry were the only lakes which showed thermal stratification and hence low bottom temperatures (4.7° C. and less) at the times of our investigations. The low readings of −0.1 and 1.3° C. listed for Manito and Bitter Lakes are a result of the lowering of the temperature of maximum density by high salinity.

TABLE I

PHYSICAL AND CHEMICAL FEATURES OF 48 LAKES IN SOUTHERN SASKATCHEWAN

Lake	Date	Area, sq. mi.	Depth, ft.	Temp., °C.		O_2, cc./liter		pH		Transparency, ft.
				Surf.	Bott.	Surf.	Bott.	Surf.	Bott.	
Antelope	30.7.38	6	28	24.5	20.6	6.0	2.3	8.9	8.9	Slight
Atten's	13.8.38	.75	27	—	—	—	—	—	—	—
Basin	30.6.39	24	35	20.2	8.6	6.5	4.8	8.7	8.7	9
Big Quill	3.7.39	162	20	18.4	17.5	6.6	—	8.8	—	2
Birch	15.6.39	15	13	14.6	14.3	6.5	5.5	8.8	8.8	3
Bitter	26.5.39	2.5	50	14.6	1.3	7.0	0.4	8.9	8.9	—
Brightsand	9.8.39	16	22	12.7	11.3	6.7	6.7	8.7	8.7	20
Broughton	2.8.39	.25	17	20.7	20.5	6.3	3.3	8.7	8.6	3
Carlyle	30.6.38	4	50	20.3	13.0	5.2	2.8	8.7	8.3	—
Christopher	26.6.39	2	33	15.1	15.0	6.6	5.6	8.7	8.7	32
Clearwater	27.7.38	.25	32	22.7	18.2	5.6	2.2	8.8	8.7	—
Deep	25.5.39	.25	78	11.3	4.5	7.8	0.9	8.5	7.4	—
Echo	30.8.39	4	57	19.7	18.4	6.5	1.5	8.7	8.5	6
Edward's	15.7.38	.75	13	21.6	21.5	4.9	4.8	8.3	8.3	—
Elbow	21.8.40	.12	70	19.0	3.7	6.0	0.5	8.3	7.5	17
Emma	27.6.39	4	30	15.8	15.1	6.3	4.9	8.7	8.7	13
Fishing	1.7.39	14	35	18.6	13.7	6.1	3.6	8.6	8.4	11
Good Spirit	5.7.38	17	13	24.2	20.7	5.8	4.9	8.7	8.6	Slight
Greenwater	1.7.39	2	25	18.6	15.6	6.2	2.0	8.4	7.9	10
Helene	15.6.39	8	18	—	—	—	—	—	—	—
Jackfish	19.8.40	29	18	17.8	16.7	6.4	6.1	8.7	8.7	5
Kenderdine	22.7.39	.25	38	19.2	13.2	7.3	0.2	8.8	8.4	8

Kenosee	29.6.38	3	25	21.0	18.1	5.2	4.0	8.6	8.4	—
Last Mountain	13.7.40	82	80	19.3	17.0	6.8	3.5	8.6	8.4	18
Lenore	17.7.38	36	23	20.7	20.2	6.8	5.6	8.9	8.9	Slight
Little Loon	14.6.39	.25	33	15.2	11.5	7.1	2.1	8.7	8.1	7
Little Manitou	17.7.40	6.5	12	20.8	17.4	6.4	0.4	8.7	8.5	8
Little Quill	2.7.39	70	5	22.6	—	6.5	—	8.7	—	Slight
Madge	8.7.38	7	43	21.7	17.2	5.2	0.6	8.2	7.7	—
Manito	31.5.39	44	75	14.4	−0.1	6.4	2.1	9.4	9.5	7
Margo	2.7.39	1	11	20.2	20.2	6.5	6.5	8.8	8.7	2.5
Meeting	5.8.39	5	43	20.7	18.2	6.4	2.3	8.5	8.0	8
Midnight	31.7.39	9	7	—	—	—	—	—	—	—
Murray	13.8.40	6	27	19.8	19.8	6.0	6.0	8.7	8.7	14
Pasqua	8.7.39	5.75	50	—	—	—	—	—	—	—
Pelletier	29.7.38	1.5	27	21.7	20.2	5.4	5.2	8.6	8.6	—
Redberry	8.9.41	28	80	16.3	4.7	6.4	0.7	8.7	8.5	10
Round (P.A.)	22.6.39	.5	20	17.4	15.4	6.5	6.0	8.6	8.5	10
Round (Qu'App.)	27.6.38	4	38	19.0	16.4	5.6	3.6	8.4	7.9	—
Sandy Beach	23.7.39	.5	40	—	—	—	—	—	—	—
Soda	21.7.39	.5	7	18.6	18.5	6.9	—	9.6	—	5
Stoney	5.9.40	1.5	18	19.2	18.9	6.4	5.6	8.9	8.8	6
Stony	3.8.39	4	10	18.4	18.2	6.1	4.8	8.9	8.8	1
Sturgeon	23.6.39	5	27	14.6	13.4	6.6	3.2	7.9	7.9	20
Turtle	9.8.40	20	45	19.1	18.5	6.1	4.4	8.6	8.4	14
Wakaw	23.5.39	5	30	11.2	10.9	6.8	6.6	8.3	8.3	—
Wilson	8.8.38	1	22	17.8	17.6	5.1	4.4	8.9	8.9	—
Witchekan	4.8.39	16	7	—	—	—	—	—	—	—

TABLE II

ANALYSES OF 41 LAKE WATERS. COMPOSITION EXPRESSED IN PARTS PER MILLION

Lake	Date	SiO_2	Al_2O_3 Fe_2O_3	Ca	Mg	K*	Na**	CO_3	HCO_3	Cl	SO_4	Salinity
Little Manitou	17.7.40	20	227	514	11160	1017	17950	236	554	23295	51720	106693
Big Quill	3.7.39	16	64	117	2467		4511	89.3	430	2097	16120	25911
Little Quill	2.7.39	16	48	131	1820		3115	70.5	382	1435	11650	18668
Manito	17.8.38	52	20	77	3280		826	992.0	1804	1552	9745	18348
Bitter	26.5.39	10	6.5	19	1192		2359	241.0	675	252	8425	13180
Redberry	13.6.40	4	9.0	71.5	1590	110	1547	96.2	483	147	9086	13144
Antelope	1.8.38	22	20.0	22.0	1132		2328	235.0	717	325	8002	12803
Basin	16.7.38	9	14.0	156.0	1308		1448	91.0	514	428	7430	11393
Stoney	1.7.40	17	12.0	121.0	1018	180	411	37.6	296	65	5009	7167
Lenore	18.7.38	21	4.8	45.7	683		577	105.8	407	153	3316	5313
Fishing	11.7.38	13.7	4.5	84.3	369		279	38.1	280	51.8	1891	3011
Wakaw	23.5.39	4.2	1.2	194.0	288		251	3.1	202	56.0	1885	2885
Last Mountain	11.7.40	14.0	5.6	44.0	167	16.6	527	34.4	364	178.0	1302	2653
Atten's	13.8.38	6.3	1.2	6.2	121.3		309	74.0	678	31.7	444	1672
Carlyle	30.6.38	9.0	2.0	34.7	211.0		83.5	71.5	411	18.8	628	1470
Margo	11.7.38	10.1	3.0	86.6	87.7		213.0	0.3	202	6.6	831	1440
Echo	6.7.40	10.0	2.4	59.9	70.8	22.4	252.0	16.0	274	105.0	594	1407
Jackfish	29.5.40	7.8	2.0	21.9	141.0	28.2	160.0	45.8	387	13.5	581	1388
Round (P.A.)	23.6.39	6.2	1.0	46.7	183.0		48.6	7.1	231	2.72	741	1267
Kenosee	30.6.38	10.5	2.3	41.8	184.0		37.4	62.3	309	18.0	587	1252
Kenderdine	20.8.38	4.2	0.8	21.0	78.3		223.0	36.7	517	9.8	346	1237
Pasqua	25.6.38	22.0	3.0	58.4	54.9		184.0	8.7	269	65.8	426	1092
Wilson	9.8.38	19.6	1.4	48.2	175.0		16.4	182.2	359	6.4	259	1067
Clearwater	29.7.38	11.2	1.8	8.9	136.0		84.0	90.0	541	20.5	134	1027
Round (Qu'App.)	27.6.38	18.0	2.5	52.6	53.5		169.0	14.5	245	58.5	395	1009
Good Spirit	7.7.38	39.4	1.4	41.3	127.0		38.6	40.5	236	7.3	420	952
Edwards	15 7.38	8.0	3.3	50.6	111.0		49.0	10.1	226	3.3	462	923
Murray	11.8.40	6.8	0.8	36.0	82.6	7.5	89.7	28.0	243	8.7	361	864
Birch	15.6.39	30.7	1.0	8.8	110.0		44.3	77.6	510	1.74	18.6	803
Christopher	26.6.39	3.4	0.58	15.3	95.2		32.7	32.9	413	1.27	101.0	695
Emma	27.6.39	6.6	0.95	18.7	89.0		36.0	30.6	398	1.97	113.0	695
Brightsand	10.6.39	13.7	1.3	11.2	80.4		38.1	39.4	422	1.6	25.9	634
Pelletier	29.7.38	9.0	0.8	23.3	68.3		50.2	38.9	326	8.7	99.7	625
Sturgeon	23.6.39	11.5	1.1	63.7	44.6		28.3	17.0	234	1.83	174.0	576
Little Loon	14.6.39	6.0	0.45	23.7	60.2		18.9	24.0	351	1.74	16.4	502
Greenwater	14.7.38	17.6	2.6	48.0	35.6		35.0	10.4	174	21.5	146.0	491
Madge	8.7.38	17.8	1.0	49.5	45.3		4.9	12.8	146	2.0	170.0	449
Deep	25.5.39	5.6	0.4	42.3	33.7		14.1	12.1	271	0.3	38.9	418
Turtle	14.6.39	8.8	1.0	23.9	30.1		27.3	9.2	254	2.0	13.9	370
Meeting	5.8.39	2.7	1.5	22.7	31.4		16.4	16.4	197	Trace	27.2	315
Helene	15.6.39	1.7	1.0	20.3	28.1		12.6	1.2	228	1.7	2.2	297

* *When not listed in this column, potassium is included with sodium.*
** *Sodium determined by difference.*

The surface waters of these lakes are liberally oxygenated (4.5 to 7.8 cc. per liter) at all times during the summer months. In shallow lakes, the more or less continuous circulation by wind ensures relatively high amounts of dissolved oxygen in bottom waters as well. Thermally stratified lakes in this area usually show an oxygen content in the hypolimnion region of less than 1 cc. per liter. In some of the other deeper lakes there is a reduction in the oxygen near the bottom but as a rule it is not severe.

All of the lakes under investigation are characterized by water having an alkaline reaction. The pH at the surface is between the limits 7.9 to 9.6, and is generally in the neighborhood of 8.6. The bottom pH is usually equal to, or only slightly less than, the surface reading except in some lakes showing a considerable degree of summer stagnation where it drops as low as 7.4.

The depth of light penetration as measured by Secchi's disk varies from 1 to 32 ft. with the majority of readings between 7 and 17 ft. Several lakes which have transparencies of 3 ft. or less are shallow and had been stirred by wind prior to examination.

Salinity

The results of water analyses show that the lakes of southern Saskatchewan exhibit a wide range of saline conditions. Data for surface samples from 41 of the lakes, with compositions expressed in parts per million, are presented in Table II. The sum of the amounts of determined inorganic constituents for each lake is given in the column headed salinity. In this study the term salinity is used to express as accurately as possible the total amount of anhydrous, inorganic material contained in one million parts of water. This differs from the customary usage in which salinity refers to the total amount of anhydrous, inorganic matter which remains after evaporation of a water to dryness. Since our method of expression takes full account of the bicarbonate content of lake waters, it is a more exact description of conditions as they occur in nature.

For each of the seven remaining lakes the sole determination was that of total residue after heating for one hour at 180° C. These values, which give a rough idea of the salinities involved, are recorded in Table III. Similar determinations are available for the other lakes and it is possible, therefore, to fit these seven into the series with a fair degree of accuracy.

The complete range of salt content for these lakes is from about three times the quantity found in sea water to the low amounts typical of fresh waters in general. Little Manitou Lake with a concentration of over 100,000 p.p.m. is extremely saline. Seven other lakes with from 10,000 to 30,000 p.p.m. are highly saline. Twenty have salinities of 1000 to 10,000 p.p.m. The remaining 20 with between 200 and 1000 p.p.m. show slight to moderately saline conditions.

Lakes with small amounts of dissolved solids are obviously freshwater and those with large quantities are saline. Any boundary between these two types must be purely arbitrary and must take account of the way in which

TABLE III

TOTAL RESIDUES FOR SEVEN LAKES

Lake	Date	Total residue* (p.p.m.)
Soda	2.6.39	9318
Elbow	21.8.40	2538
Sandy Beach	23.7.39	1547
Witchekan	4.8.39	838
Midnight	31.7.39	448
Stony (nr. Turtle)	3.8.39	412
Broughton	2.8.39	171

* *Evaporated for one hour at 180° C.*

mineral analyses are reported. Hesse (20) and Welch (43) have suggested a useful classification in which the dividing line is placed at 300 p.p.m. of dissolved solids (this limit is based presumably on analyses in which bicarbonate is included with carbonate). According to such a scheme Deep, Turtle, Meeting, Helene, and Broughton would be considered freshwater lakes and the rest saline in nature.

There is a definite correlation between the geographic location of a lake and its salinity. All of the lakes in the prairie and parkland show a well-developed salinity which is usually above 1000 p.p.m. Lakes in the forest zone tend to be fresh in nature. Some of those in its southern portion exhibit a moderate degree of salinity and Bitter Lake, which has a high salt concentration, is quite atypical.

A significant difference in the composition of the waters in these two regions is noticed also. Thus, with few exceptions, lakes of the prairie and parkland contain a preponderance of sulphate, sodium, and magnesium whereas in those of the forested region, bicarbonate, calcium, and magnesium are the main constituents. The former may be described as sulphate types and the latter as carbonate types (see Table XII in appendix for compositions expressed as percentages of salinities). Such dissimilarities in concentration and composition may be explained largely on the basis of difference in the climatic conditions, soil, and vegetation of these regions (38).

Both long term and seasonal changes in mineral content are observed in the saline lakes of this area. Available information indicates that during the period since 1920 the concentration has increased in different lakes by amounts ranging from 20 to 100%. The data suggest also that there was a marked increase in the rate of accumulation of salts in the years from 1937 to 1941. Seasonal changes usually follow a definite pattern which involves a reduction in salt content as a result of the spring runoff and then an increase during the summer months. The variations are of small magnitude in lakes having low concentrations, but in the more saline waters variations commonly reach magnitudes of 5 to 8%. Still more pronounced changes are evident in such

highly saline waters as Redberry Lake. Here the total residue of surface water was 14,410 p.p.m. on September 24, 1940 whereas between May 9 and August 12 of 1941 it increased from 13,639 to 14,946 p.p.m.

It is also noted that as salinity increases, there is a decrease in the relative concentration of calcium and carbonates with an increase in those of sodium, magnesium, and sulphate. Such conditions are a result of differences in the relative solubilities of the salts formed by these constituents. Increasing concentration will first result in the precipitation of less soluble salts such as calcium carbonate and eventually may also cause the deposition of those like sodium sulphate which are somewhat more soluble. The latter condition is exemplified in Little Manitou Lake where an extensive bed of Glauber's salt is found.

Distribution of Entomostraca

The following section contains a list of the Entomostraca collected in southern Saskatchewan along with some notes on their relative numbers and distribution in this area. The classification and arrangement of species is based on the works of Pearse, Birge, and Marsh as presented in Ward and Whipple (37, 9, and 30). Brief references to the occurrence of these species in saline lakes of other regions have been included.

Anostraca

Artemia salina (Linnaeus). Collected in only one lake, Little Manitou, which is the most saline of the series. This 'brine shrimp' is the dominant member of the fauna in the lake and at times its abundance is indicated visibly by the reddish color imparted to the water. It has been reported from highly saline waters in Connecticut, Utah, and California, as well as from many other parts of the world.

Other phyllopods are known to occur in this region in various temporary bodies of water which, however, were outside the scope of this study.

Cladocera

Sida crystallina (O. F. Müller). Found in the littoral samples from four lakes with salinities of 864, 370, 315, and 297 p.p.m.

Diaphanosoma leuchtenbergianum Fischer. Widely distributed, occurring in the limnetic samples of 28 lakes. It was common in salt concentrations up to 7167 and very rare in 13,144 p.p.m. Birge (9) suggests that this species may be merely a variety of *Diaphanosoma brachyurum*. The latter has been recorded by Young (48) in three North Dakota lakes having total solids in the neighborhood of 13,000 p.p.m. and by Beadle (6) from one locality with a density of 1.005.

Daphnia magna Straus. Taken in Lenore and Margo Lakes which showed salinities of 5313 and 1440 p.p.m. This species has been reported from slightly brackish waters in Europe (9) and from three Algerian waters with salt contents up to at least 8200 p.p.m. (6).

Daphnia pulex (de Geer). Occurred commonly in the collections from 17 widely scattered lakes with salt concentrations ranging from 13,144 to 449 p.p.m. Hutchinson (23) reports this species in Pyramid Lake, Nevada, with dissolved materials of approximately 3500 to 4000 p.p.m.

Daphnia longispina (O. F. Müller). The most widely distributed entomostracan being found in 41 of the 48 lakes. It occurred throughout the whole salinity range and was the only member of the Cladocera in the three most saline lakes. Although rare in Little Manitou Lake, this species was abundant in lakes of lower salinity. In North Dakota, *D. longispina* was taken occasionally in a lake with total solids of 13,462 p.p.m. (48).

Simocephalus vetulus (O. F. Müller). Taken rarely in the littoral samples from three lakes having salt contents of 7167, 864, and 370 p.p.m. Recorded once by Young (48) where the total solids were somewhat less than 13,462 p.p.m.

Scapholeberis mucronata (O. F. Müller). Found occasionally in littoral collections from Murray and Turtle Lakes (864 and 370 p.p.m.) while a single specimen was taken from Redberry Lake (13,144 p.p.m.).

Ceriodaphnia reticulata (Jurine). Taken only in Turtle Lake (370 p.p.m.) where it was occasional in the limnetic samples. Hutchinson (22) in a table showing distributions of some Daphniidae in various parts of the world lists this species from a salt concentration of approximately 1400 p.p.m.

Ceriodaphnia lacustris Birge. Common in the open water samples of 12 widely separated lakes with salinities between 3011 and 315 p.p.m.

Ceriodaphnia quadrangula (O. F. Müller). Also widely distributed and of occasional abundance in 15 lakes with salt contents ranging from 13,180 to 491 p.p.m.

Moina hutchinsoni Brehm. Collected from Manito Lake (18,348 p.p.m.) where it occurred in both open-water and near-shore samples. The only previous records of this species appear to be those of Hutchinson (23) who reports it from Winnemucca and Big Soda Lakes in Nevada. Using data from Hutchinson and from Clarke (13) the total solids of these lakes at the time of Hutchinson's investigations have been estimated at approximately 50,000 and 22,500 p.p.m. respectively. This species closely resembles *Moina macrocopa* Straus and since it has only recently been described (11) it is quite likely that the same form has been determined as *M. macrocopa* in earlier studies.

Bosmina obtusirostris Sars. Occurred rarely in Redberry (13,144), Basin (12,803) and Soda (total residue 9318 p.p.m.) but was quite common in the limnetic samples of 37 lakes having lower salinities.

The next 16 species were confined to the littoral regions of the lakes indicated.

Bosmina longispina Leydig. Rare in Sturgeon Lake (576 p.p.m.).

Ilyocryptus acutifrons Sars. Rare in Turtle Lake (370 p.p.m.).

Macrothrix laticornis (Jurine). Rare in Wilson Lake (1067 p.p.m.).

Eurycercus lamellatus (O. F. Müller). Was rare in Round, Murray, and Turtle Lakes (1267, 864, and 370 p.p.m.).

Camptocercus rectirostris Schoedler. Taken rarely in Echo (1407 p.p.m.), Round (1267 p.p.m.), and Emma (695 p.p.m.) Lakes.

Acroperus harpae Baird. Rare in samples from Madge (449 p.p.m.) and Turtle (370 p.p.m.) Lakes.

Leydigia quadrangularis (Leydig). Rare in Carlyle and Wilson Lakes (1470 and 1067 p.p.m.).

Alona guttata Sars. Found rarely in collections from Atten's, Emma, and Turtle Lakes (1672, 695, and 370 p.p.m.).

Alona affinis (Leydig). Was present rarely in Fishing (3011 p.p.m.) and Last Mountain (2653 p.p.m.) Lakes.

Alona costata Sars. Taken occasionally in Last Mountain, Jackfish, Murray, Stony, Greenwater, and Turtle Lakes with salinities from 2653 to 370 p.p.m.

Alona rectangula Sars. Of occasional abundance in samples from 13 lakes having salinities of 2885 p.p.m. (Wakaw) and less. Young (48) records this species in water having total solids approaching 13,462 p.p.m. Beadle (6) mentions that *A. rectangula* has been reported by Gauthier (17) from coastal and inland saline waters of Algeria. Beadle himself found it in waters with densities of 1.002 and 1.008.

Graptoleberis testudinaria (Fischer). Rare in Jackfish and Turtle Lakes (1388 and 370 p.p.m.).

Alonella nana (Baird). Occurred only in Turtle Lake (370 p.p.m.) where it was rare.

Pleuroxus denticulatus Birge. Found rarely in Redberry Lake (13,144 p.p.m.) and occasionally in six other lakes of moderate to low salinity (1285 to 461 p.p.m.). This species is widely distributed throughout the area.

Pleuroxus aduncus (Jurine). Taken rarely in Fishing, Wakaw, and Kenosee Lakes (3011, 2885 and 1252 p.p.m.).

Chydorus gibbus Lilljeborg. Was of rare occurrence in Murray (864 p.p.m.) and Turtle (370 p.p.m.) Lakes.

Chydorus sphaericus (O. F. Müller). One of the most frequent cladocerans in the limnetic regions of lakes in southern Saskatchewan. It was rare in Redberry (13,144 p.p.m.) and Basin (11,398) p.p.m.) Lakes but common in 34 others of lower salinities. Young (48) mentions one record of this species in water having total solids of about 13,462 p.p.m.

Polyphemus pediculus (Linné). Collected rarely in the littoral zones of Murray and Turtle Lakes (864 and 370 p.p.m.).

Leptodora kindtii (Focke). Was of rare occurrence in the limnetic samples from 10 widely scattered lakes having salinities of 3011 p.p.m. and less.

Copepoda

Diaptomus oregonensis Lilljeborg. Found in six lakes all of which lie in the northern part of the area. The salinities ranged from about 2500 to 250 p.p.m. Marsh (31) reports *D. oregonensis* from Kinistino, Saskatchewan

(35 miles southeast of Prince Albert). The species is widely distributed across the central part of North America but does not appear to be an inhabitant of saline lakes except in Saskatchewan.

Diaptomus tenuicaudatus Marsh. The most widespread species of *Diaptomus*. It was collected from the limnetic regions of 27 lakes with salinities of 18,668 to 297 p.p.m. *D. tenuicaudatus* was described from material gathered in Glen Lake, Saskatchewan and has also been recorded from Lake Nipigon, Ontario (31) and from a single lake in each of the States of Washington (27) and Nevada (23). A closely related species, *Diaptomus sicilis*, was reported by Willey (45) as the most abundant entomostracan in collections made in the Quill Lakes in 1920. The writer has examined a sample of this material and considers that the *Diaptomus* present therein is *tenuicaudatus* rather than *sicilis*.

Diaptomus shoshone Forbes. This large copepod occurred in the limnetic samples of Redberry, Antelope, Soda, and Last Mountain Lakes and thus had a salinity range of 13,144 to 2653 p.p.m. Young (48) reported this species in three North Dakota lakes having total solids up to 16,165 p.p.m. while Bajkov recorded it for Manito Lake in 1927 when the total solids were 15,530 p.p.m. (4).

Diaptomus ashlandi Marsh. Taken only in Deep Lake which lies in the forest zone and has a salinity of 418 p.p.m.

Diaptomus siciloides Lilljeborg. Occurred in 13 lakes of the prairie and parkland regions and was the species of *Diaptomus* showing the widest salinity range. Although rare in Little Manitou Lake it was common and often abundant in the remaining lakes which had salt contents from 25,911 to 625 p.p.m. This species was reported by Young (48) in three lakes with total solids up to 25,450 p.p.m.

Diaptomus sp. The samples from Clearwater and Wakaw Lakes contained immature specimens of *Diaptomus* of undetermined species.

Cyclops viridis Jurine. Was widely spread through the area occurring in 32 lakes with salinities from 18,668 to approximately 200 p.p.m. Beadle (6) found this species in water having a salinity of about 68,000 p.p.m., Young (48) recorded it from Devil's Lake (total solids 15,210 p.p.m.), and Schäfer (40) reported it from a salt concentration of 7250 p.p.m. Both Schäfer and Gurney (18) have mentioned the occurrence of *C. viridis* in brackish waters of Europe.

Cyclops bicuspidatus Claus. Was the most widely distributed copepod. It was very rare in Little Manitou Lake but common in 35 other lakes ranging in salinity from about 9000 to 297 p.p.m. Schäfer (40) has recorded the species from waters of 5620, 6030, 8830 and 15,890 p.p.m. while Florentin (15) found *C. bicuspidatus* living in 3% salt solutions (30,000 p.p.m.) in Lorraine.

Cyclops leuckarti Claus. Like the two preceding species was found in all areas but its occurrence was limited to 22 lakes having salinities less than 3000 p.p.m. Young (48) recorded *C. leuckarti* from one lake with total solids in the neighborhood of 19,000 p.p.m. and Annandale (2) has reported it from

brackish ponds in Bengal where the salt contents were between 23,160 and 12,130 p.p.m. Sewell (41) has also found this species in salinities varying from 170 to 4380 in waters of the Calcutta region.

Cyclops albidus Jurine. Was taken rarely in the littoral collections from nine widely scattered lakes, the salinities of which ranged from 2653 to 370 p.p.m.

Cyclops serrulatus Fischer. A widely distributed species typical of the near-shore regions. It was exceedingly rare in Redberry Lake but of occasional abundance in 20 other lakes with salinities from 3011 to 297 p.p.m. Young (48) has recorded this species from Devil's Lake when the total solids were somewhat less than 13,462 p.p.m.

Cyclops fimbriatus Fischer. Occurred in small numbers in the littoral collections from Atten's, Emma, Murray, and Turtle Lakes (1672 to 370 p.p.m.) all of which lie in the parkland and forest zones. Beadle (6) found this species in a desert well with a salinity of about 8200 p.p.m. and Gurney (18) has referred to its presence in saline waters of Europe.

Cletocamptus albuquerquensis (Herrick). Was taken in Little Manitou, Big Quill, and Little Quill Lakes, all of high salinity (106,693 to 18,668 p.p.m.). This species was described by Herrick (19) under the name *Marshia albuquerquensis*. Although he did not indicate the locality of collection, Marsh in Ward and Whipple (1918, p. 780) gives it as fresh and brackish waters of New Mexico. Under the same name it has been recorded by Young (48) in five lakes with total solids from 62,929 to 14,603 p.p.m. as well as from fresh water. Hutchinson (23) collected *C. albuquerquensis* in Winnemucca and Big Soda Lakes, Nevada, when the total solids were approximately 50,000 and 22,500 p.p.m. respectively.

Laophonte mohammed Blanchard and Richard. Found occasionally in the littoral samples from eight lakes ranging in salinity from 13,144 to 864 p.p.m. *L. mohammed* is an inland water representative of a typically marine genus and has been recorded from freshwater as well as saline lakes in several parts of the world. Beadle (6) reported it from water of density 1.052. Schäfer (40) found it in salt concentrations of 15,890 and 7800 p.p.m. and Young (48) collected it in a lake with total solids approximately 9000 p.p.m. Willey (46) gives the characteristics which separate *L. mohammed* and *C. albuquerquensis*, both of which are very minute species.

Canthocamptus dentatus Poggenpol. Was present in the littoral regions of Jackfish (1388 p.p.m.), Sturgeon (576 p.p.m.), and Turtle (370 p.p.m.) Lakes. Kemmerer *et al.* (27) have reported this species under the name *Canthocamptus northumbricus* from Bear Lake, Idaho, which had a salinity of 1060 p.p.m.

Ostracoda

Ostracods were found occasionally in the littoral samples of all lakes except the four having the highest salinities, but this difficult group received little attention in the present study. *Lymnicythere reticulata* Sharpe was taken in Redberry, Basin, Soda, Wilson, and Atten's Lakes (13,144 to 1672 p.p.m.).

Cypris sp. occurred in Redberry, Last Mountain, Sandy Beach, Kenosee, Clearwater, Stony, and Helen Lakes (13,144 to 297p .p.m.) while *Candona* sp. was present in Redberry Lake.

Salinity and Distribution

As revealed in the foregoing notes on occurrence some species of Entomostraca showed a general distribution, others were found in a small number of lakes often widely separated and still others were recorded from only a single lake. A similar condition has been noted by other observers who have made surveys of this type, and it is generally recognized that considerable caution must be exercised in making statements concerning geographic distribution on the basis of restricted collections. This is particularly true of the plankton organisms of inland lakes since certain forms, for reasons which are not yet completely understood, may be abundant at one time and scarce or apparently absent at another.

In this study most of the species which were found in a single lake or in only a few lakes occurred in the weedy, near-shore regions and it is quite possible that their limited distribution may be partly a result of insufficient sampling in this type of habitat. For this reason, the main discussion of littoral forms will be centered on distribution in those lakes which were examined most thoroughly. Our extensive collections showed that during the summer months the limnetic Entomostraca remain unchanged as to species from year to year and hence a more particular treatment of these forms is possible.

Our data suggest that, of all the physical and chemical factors involved, salinity is predominant in controlling the distribution of life in these lakes. The importance of salt content as a lethal factor for aquatic organisms has been recognized by many workers and it is the general belief that both the amount and composition of dissolved salts are of significance in the production of salinity effects. A consideration of relative concentrations of various ions also involves the phenomena of ionic antagonism and this field, for the most part, has been neglected in studies of saline environments.

From the very interesting work of Martin and Wilbur (32) and more especially that of Boone and Baas-Becking (10) it is evident that potassium chloride by itself is highly toxic to *Artemia salina*. Boone and Baas-Becking have shown also that the toxic effect of potassium chloride may be antagonized to a small degree by sodium and to a lesser extent by magnesium and calcium. These investigators point out that the absence of *Artemia* from desert lakes rich in potassium fits in well with their laboratory findings.

In the same paper these authors deal with the factors responsible for the disappearance of *Artemia* from salt evaporating basins, as evaporation proceeds and suggest that the increasing magnesium content, together with a sudden depletion of the food supply, is of chief importance in this regard. From their discussion and from a table showing concentrations of cations as sea water evaporates (3, p. 766) it can be seen that the molarity of magnesium when *Artemia* disappears is in the neighborhood of 0.3. That such a concentration in itself is not lethal is shown by existence in Little Manitou Lake where the

magnesium content is at least 0.46 molar. This observation indicates the need for further studies on the nature of antagonisms in concentrated solutions —a point that is recognized by Boone and Baas-Becking—and also suggests that the loss of food supply seems a more probable explanation of the case in question.

The natural environment of *Artemia salina* is considered usually to be highly saline water containing a preponderance of sodium chloride, whereas in Little Manitou Lake this species is living where sodium and sulphate are the main constituents with magnesium and chloride being present in lesser, but still relatively high amounts. Beadle (6) records *A. salina* from an Algerian lake which has a chemical composition quite similar to that of Little Manitou Lake. *Artemia* was present in Big Soda Lake, Nevada, when sodium, chloride, and carbonate showed high concentrations (13 and 39). Thus while *Artemia* can live in a wide variety of saline habitats it may be noted that these always contain large quantities of sodium and chloride. This may suggest that as long as the sodium and chloride contents are adequate, the concentrations of other ions, aside from potassium, are relatively unimportant.

Although several authors have stated that variations in the amounts of different ions are of considerable significance in determining the distribution of Entomostraca, very little appears to be known about this subject or about the ionic balance required by forms other than *Artemia salina*. Hutchinson (22) has investigated the magnesium tolerance of six species of Daphniidae, including *Daphnia longispina*, *D. pulex*, and *D. magna* in experiments utilizing magnesium chloride solutions. In southern Saskatchewan all of these species occurred in lakes with magnesium concentrations which greatly exceeded those determined as upper limits. Hutchinson noted a like discrepancy between experimental upper limits and natural distribution and remarked that ionic antagonism or the existence of salt tolerant races might be involved in an adequate explanation of the facts. The possibility of races which tolerate different degrees of ionic constituents is indicated by the observation that one of his two clones of *D. longispina* had an upper limit which was twice as great as that of the other.

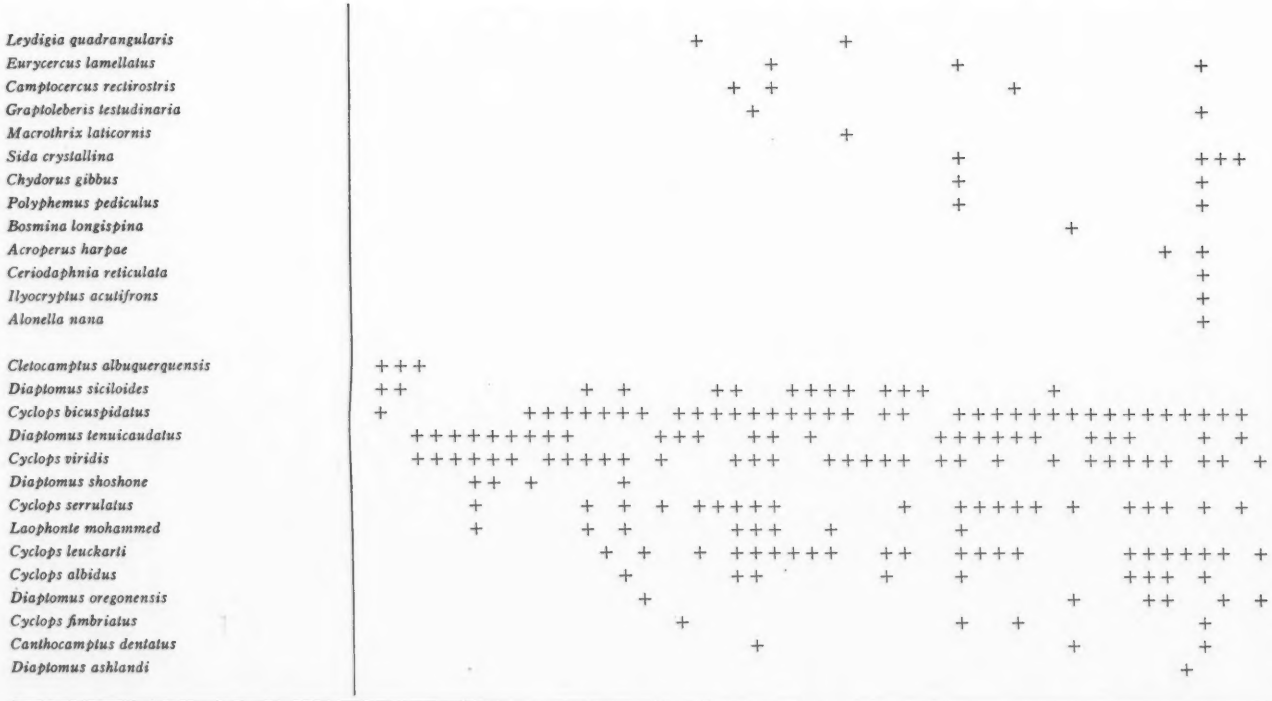
The low calcium in the most saline lakes might be responsible for a decrease in the number of species but it should be pointed out that the absolute concentration is higher than in many lakes which contain a wide variety of Entomostraca. There is, as suggested by Beadle (6), the possibility that a low relative calcium content is a limiting factor for some organisms but this does not appear to be indicated by our studies.

On the basis of this investigation, there appears to be nothing to show that there are correlations between the concentrations of individual ions and the distribution of Cladocera, Copepoda, and Ostracoda. Total salinity, then, is assumed to be the predominant factor in these lakes. If total salinity is a dominant factor, then the wide range of concentrations encountered in this study should be correlated, with pronounced differences in the distribution of Entomostraca. In the following sections, this thesis is developed.

TABLE IV

DISTRIBUTION OF CLADOCERA AND COPEPODA IN 48 LAKES OF SOUTHERN SASKATCHEWAN. LAKES IN ORDER OF DECREASING SALT CONTENT

Species of Cladocera and Copepoda	Little Manitou 106693	Big Quill 25911	Little Quill 18668	Manito 18348	Bitter 13180	Redberry 13144	Antelope 12803	Basin 11398	Soda 9318*	Stoney 7167	Lenore 5313	Fishing 3011	Wakaw 2885	Last Mountain 2653	Elbow 2538*	Sandy Beach 1547*	Atten's 1672	Carlyle 1470	Margo 1440	Echo 1407	Jackfish 1388	Round (Prince Albert) 1267	Kenosee 1252	Kenderdine 1237	Pasqua 1092	Wilson 1067	Clearwater 1027	Round (Qu'Appelle) 1009	Good Spirit 952	Edward's 923	Witchekan 838*	Murray 864	Birch 803	Christopher 695	Emma 695	Brightsand 634	Pelletier 625	Sturgeon 576	Midnight 448*	Stony 412*	Little Loon 502	Greenwater 491	Madge 449	Deep 418	Turtle 370	Meeting 315	Helene 297	Broughton 171*
Daphnia longispina	+	+	+	+	+	+	+	+			+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+			+	+	+	+	+	+	+	+	+			+	+	+	+	+	+	+	+
Moina hutchinsoni				+																																													
Diaphanosoma leuchtenbergianum						+					+	+	+		+	+			+	+	+		+		+	+	+	+	+			+	+		+	+	+			+			+			+	+	+	+
Daphnia pulex						+	+		+	+							+			+	+					+		+				+	+	+					+	+	+	+			+				+
Ceriodaphnia quadrangula					+	+					+	+		+	+		+			+	+		+					+				+									+							
Bosmina obtusirostris					+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+	+	+	+	+	+			+	+	+	+	+	+	+	+
Chydorus sphaericus					+	+		+		+		+	+	+		+	+	+	+	+	+	+	+	+	+				+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pleuroxus denticulatus					+															+			+	+			+				+	+			+														
Scapholeberis mucronata					+																											+																	
Simocephalus vetulus										+																						+																	
Daphnia magna											+								+																															
Ceriodaphnia lacustris												+		+		+					+			+	+	+	+															+				+			
Alona affinis												+	+																																				
Pleuroxus aduncus												+	+										+																										
Leptodora kindtii												+		+						+	+				+				+				+												+	+			
Alona rectangula													+			+	+		+			+											+		+	+	+		+								+	+	
Alona costata														+							+												+																
Alona guttata																	+																	+															



[a] *Total residues. All other figures are salinities.*

Total Salinity and Distribution of Cladocera, Copepoda, and Ostracoda

Table IV contains the records of Cladocera and Copepoda with the species arranged in order of salinity tolerance and the lakes in order of decreasing concentration.

Cladocera

Included among the widely distributed Cladocera were all the limnetic forms except *Daphnia magna* and *Ceriodaphnia reticulata* in addition to three species characteristic of the littoral region. The limnetic forms were *Daphnia longispina*, *Ceriodaphnia quadrangula*, *Diaphanosoma leuchtenbergianum*, *Daphnia pulex*, *Bosmina obtusirostris*, *Chydorus sphaericus*, *Ceriodaphnia lacustris*, and *Leptodora kindtii* while *Pleuroxus denticulatus*, *Alona rectangula*, and *Alona costata* were the littoral forms in question.

Daphnia longispina was found throughout the entire range of salinities and thus showed the greatest tolerance. Moreover, it was the sole cladoceran in the three most saline lakes. The next five species along with *P. denticulatus* occurred in lakes in which the concentrations were below 14,000 p.p.m. *C. lacustris*, *L. kindtii*, *A. rectangula*, and *A. costata* were present in lakes with salt contents of approximately 3000 p.p.m. and below.

Each of the remaining 20 species was collected in four lakes or less and since all except *Daphnia magna*, *Ceriodaphnia reticulata*, and *Moina hutchinsoni* were littoral forms, with distribution possibly affected by insufficient sampling, they will be referred to only briefly. *M. hutchinsoni* occurred in a salinity of 18,348 p.p.m. while in Nevada it was found only in still higher concentrations. Its known distribution indicates a preference for highly saline waters and it is the only cladoceran of which this is true. It is apparently closely allied in habits to *M. salinarum* which is a halophil species in Algeria (6). *Scapholeberis mucronata* was present in salinities up to 13,144 p.p.m., *Simocephalus vetulus* tolerated 7167 and *Daphnia magna* 5313 p.p.m., *Alona affinis* and *Pleuroxus aduncus* occurred in concentrations up to 3011 p.p.m., while *Alona guttata*, *Leydigia quadrangularis*, *Eurycercus lamellatus*, *Camptocercus rectirostris*, *Graptoleberis testudinaria*, and *Macrothrix laticornis* were confined to salinities of less than 2000 p.p.m. *Sida crystallina*, *Chydorus gibbus*, *Polyphemus pediculus*, *Bosmina longispina*, and *Acroperus harpae* were restricted to lakes of less than 1000 p.p.m. and *Ceriodaphnia reticulata*, *Ilyocryptus acutifrons*, and *Alonella nana* were taken in 370 p.p.m. only.

Copepoda

Among the Copepoda a widespread distribution was noted in the case of the limnetic species *Diaptomus siciloides*, *Cyclops bicuspidatus*, *Diaptomus tenuicaudatus*, *Cyclops viridis*, *Cyclops leuckarti*, and also for the littoral forms *Laophonte mohammed*, *Cyclops serrulatus*, and *Cyclops albidus*. The greatest salinity tolerance was shown by the first two species which were found, although very rarely, in the extremely saline water of Little Manitou Lake.

D. tenuicaudatus and *C. viridis* stood next in this respect and occurred in concentrations up to 18,668 p.p.m. *L. mohammed* and *C. serrulatus* were confined to lakes having salt contents of 14,000 p.p.m. and less, whereas *C. leuckarti* and *C. albidus* were found in salinities of less than 3000 p.p.m. It is of interest to note that while only one widely distributed cladoceran was present in concentrations above 14,000 p.p.m., there were four copepods of which this was true.

The copepods which occurred in only a few lakes included the limnetic species *Cletocamptus albuquerquensis*, *Diaptomus shoshone*, *Diaptomus oregonensis*, and *Diaptomus ashlandi*, as well as the littoral forms *Cyclops fimbriatus* and *Canthocamptus dentatus*. *C. albuquerquensis* is noteworthy owing to its restriction to the three lakes showing the greatest salinities. It might be considered a strictly halophil species were it not for its presence in freshwater habitats elsewhere. Other species such as *C. retrogressus* Schmankewitsch and *C. confluens* (Schmeil) also tolerate a wide variety of saline conditions (6, 18, and 41). *D. shoshone* was found in concentrations up to 14,000 p.p.m. while *D. oregonensis* was confined to those of about 2500 p.p.m. and less. *C. fimbriatus* and *Canthocamptus dentatus* were taken in lakes having salt contents below 2000 p.p.m., whereas *D. ashlandi* occurred only in the slightly saline Deep Lake (418 p.p.m.).

Ostracoda

As this group was not made the subject of intensive study, very little can be said about the salinity tolerance of its members. *Lymnicythere reticulata*, *Cypris* sp., and *Candona* sp. were found in concentrations up to 14,000 p.p.m. No ostracods were collected in higher salinities. Hence their distribution appears to be comparable to that of other freshwater Entomostraca characteristic of the littoral region.

The over-all picture of distribution in the series of lakes shows a decrease in the number of species correlated with increasing salt concentration. The

TABLE V

Number of species of Entomostraca, exclusive of Ostracoda, in eight selected lakes

Lake	Salinity, p.p.m.	Number of species				
		Cladocera		Copepoda		Total
		Limnetic	Littoral	Limnetic	Littoral	
Turtle	370	6	12	4	4	26
Murray	864	7	8	4	4	23
Jackfish	1388	6	2	4	4	16
Echo	1407	7	2	4	3	16
Last Mountain	2653	6	2	4	3	15
Stoney	7167	5	1	3	0	9
Redberry	13,144	6	2	3	2	13
Little Manitou	106,693	1	0	3	0	5*

* *Includes the anostracan Artemia salina.*

most complete information on this subject is provided by studies in eight lakes which were selected for special investigation in 1940. These data are presented in Table V. In a salinity range from 370 to 106,693 p.p.m. the total number of species of Entomostraca, excluding the Ostracoda, dropped from 26 to 5. It may be noted that the major decreases occurred at the levels of approximately 1000, 3000 and 14,000 p.p.m. Both cladocera and Copepoda were involved in the reductions in numbers but to markedly different extents. The numbers of cladoceran species showed a drop from 18 in the lowest to 1 in the highest concentrations while for the copepods the decrease was from 8 to 3 under similar conditions. These observations suggest that the Cladocera are more severely restricted by increasing salinity than are the Copepoda. A consideration in terms of limnetic and littoral forms indicates a further significant point. In these lakes the total number of limnetic species dropped from 10 to 4 while for the littoral species there was a reduction from 16 to 0. It is obvious, then, that littoral forms suffer a proportionately greater restriction than do those which are limnetic in habit.

Essentially the same features are found when the distribution of Cladocera and Copepoda in the whole series of lakes is considered. Thus, as shown in Table VI, a total of 37 species were found in lakes having salt contents up to 1000 p.p.m. and in concentrations between 1000 and 3000 p.p.m. almost an equal number (32) were present. In salinities from 3000 to 14,000 p.p.m. there was a reduction to 17 while above 14,000 p.p.m. only 7 species occurred. The number of cladoceran species showed a drop from 27 to 2 and the copepods from 12 to 5 in the total salinity range. Also the total number of limnetic species dropped from 16 to 7 while in the case of littoral species there was a reduction from 21 to 0.

The above interpretation takes cognizance of all species no matter what their salinity distributions and as may be expected, gives a somewhat distorted view of the importance of increasing salinity in restricting the occurrence of typically freshwater forms. It is known, for example, that 11 forms appeared only in salt contents below 1000 p.p.m. while another 6 were confined to the 1000 to 3000 range. Furthermore, 2 species occurred in less than 1000 p.p.m.

TABLE VI

TOTAL SALINITY AND NUMBER OF SPECIES OF CLADOCERA AND COPEPODA

Salinity, p.p.m.	Number of species						
	Cladocera		Copepoda		Cladocera and Copepoda		
	Limnetic	Littoral	Limnetic	Littoral	Limnetic	Littoral	Total
Up to 1000	9	16	7	5	16	21	37
1000 – 3000	9	11	7	5	16	16	32
3000 – 14,000	7	3	5	2	12	5	17
Above 14,000	2	0	5	0	7	0	7

TABLE VII

NUMBER OF SPECIES OF CLADOCERA AND COPEPODA FOUND IN LESS THAN 1000 P.P.M. AND THEIR REDUCTION IN HIGHER SALINITIES

Salinity, p.p.m.	Number of species						
	Cladocera		Copepoda		Cladocera and Copepoda		
	Limnetic	Littoral	Limnetic	Littoral	Limnetic	Littoral	Total
Up to 1000	9	16	7	5	16	21	37
1000 – 3000	8	7	6	5	14	12	26
3000 – 14,000	6	3	4	2	10	5	15
Above 14,000	1	0	4	0	5	0	5

and again in the 3000 to 14,000 range but were not taken in the intermediate concentrations. Considering only those species found in lakes with less than 1000 p.p.m. and tracing them through the series (Table VII) shows the influence of salinity to be still more pronounced than indicated above. Of the 25 species of Cladocera found in 1000 p.p.m. and less, only 1 occurred in salinities above 14,000 p.p.m. Similarly, only 4 out of 12 Copepoda were left in the highest range. Here again, the major change is the result of the loss of littoral species.

Species Composition of Individual Lakes

As shown in previous sections, the degree of salt concentration has a more or less progressive effect in limiting the distribution of Entomostraca in these lakes. Moreover, its influence varies according to the species, being very pronounced in some cases and of much less importance in others. These conditions are reflected in the nature of plankton communities. The communities in lakes of low to moderate concentration obviously differ in several respects but on the whole are sufficiently alike to be regarded as one large group; the faunal composition of highly saline water, on the other hand, stands in marked contrast to this and may be considered to constitute a second type of community.

COMMUNITIES IN LAKES OF LOW TO MODERATE SALINITY

A typical community of the open water of lakes with salinities below 14,000 p.p.m. consisted regularly of members of the genera *Daphnia*, *Diaphanosoma*, *Ceriodaphnia*, *Bosmina*, *Chydorus*, *Diaptomus*, and *Cyclops*. This was supplemented by the presence of *Leptodora* in several of the larger lakes having concentrations below 3000 p.p.m. The littoral fauna was more varied in composition with the number of species increasing steadily as salinity decreased. The characteristic forms in the series as a whole were *Pleuroxus*, *Alona*, *Cyclops*, and *Laophonte*. It is of interest to note that the Entomostraca in these lakes consisted exclusively of freshwater organisms with the Cladocera comprising the major portion of the fauna in nearly all instances.

A study of the distributional records reveals that many of the genera are represented by only one species in these lakes. Some, such as *Daphnia*, *Ceriodaphnia*, *Pleuroxus*, *Alona*, *Bosmina*, *Chydorus*, *Diaptomus*, and *Cyclops* have two or more species but in general only one species is found in any individual lake. In each of *Bosmina* and *Chydorus* where two species occurred in a single lake the forms involved were inhabitants of either the limnetic or the littoral regions, not of both. It would appear that each major habitat in a lake and often each lake itself acts for one species as a "niche", the occupation of which prevents the establishment of other species of the same genus. Some prominent exceptions were in evidence among closely related species of both Cladocera and Copepoda. These involved limnetic as well as littoral forms.

The genus *Daphnia* was represented by the limnetic species *pulex* and *longispina* in each of nine lakes, while in one additional lake a third species, *magna*, was also associated with them. In the case of *Alona* there were five lakes in which two species occurred in the littoral regions. Further instances of similar associations were noted for species of *Diaptomus* and for both limnetic and littoral forms of *Cyclops*. The usual condition for *Diaptomus* was to find a single species, generally *tenuicaudatus*. However, *tenuicaudatus* and *siciloides* were found together in one lake while the large *D. shoshone* which was taken in four lakes was associated with *tenuicaudatus* in three and with *siciloides* in the other. *Cyclops* stood in marked contrast to *Diaptomus* in that it was represented by two or more species in a total of 37 lakes. All six species were present in two of the lakes and there were five species in six other waters.

The coexistence of species belonging to the same genus undoubtedly involves a number of features including variations in food habits and life cycles of the different species in that genus. Although our data are not sufficient for a thorough treatment of this subject, there is one aspect which seems worthy of consideration. Carl (12) in a study of the Cladocera and Copepoda of British Columbia noted that, among the Copepoda of the family Centropagidae and Cladocera of the genus *Daphnia*, two or more associated species often differed markedly as to size. Using measurements as given in Ward and Whipple (1918) and our records, it is evident that similar relationships are exhibited in the lakes of southern Saskatchewan. Thus, *D. siciloides* and *D. tenuicaudatus* which are of comparable lengths (about 1.2 mm. for females) were associated only once whereas *D. shoshone* which occurred in conjunction with each of these species is much the largest of the three (3.0 mm. for female). Also, the littoral species of *Cyclops* which were found together in three lakes show considerable variations in their lengths (females of *C. albidus*, *C. serrulatus*, and *C. fimbriatus* are approximately 2.0, 0.8 to 1.25, and 0.5 mm. respectively). Finally, associated species of *Daphnia* have carapace lengths of about 5 mm. for *magna*, 3 mm. for *pulex*, and 1.2 mm., for *longispina*. Such size variations among closely related organisms indicate hereditary or environmental differences, or both, and they may perhaps be manifestations of dissimilar food

habits as suggested by Carl. There is a definite correlation between body size and association in the above instances but not in others such as the limnetic species *Cyclops* and the species of *Alona*.

Hutchinson (23) and Hutchinson, Pickford, and Schuurman (24) have referred to a peculiar association which occurs in certain argillotropic waters of the United States and South Africa. This consists primarily of *Daphnia pulex* along with two species of *Diaptomus*, one much larger than the other. It is of interest to note that Antelope Lake contained *Daphnia pulex*, *Diaptomus shoshone*, *Diaptomus tenuicaudatus*, and a few specimens of *Cyclops viridis* and, moreover, that it was very muddy at the time of our investigation. In the light of present information we are not justified in attributing the faunal condition of this lake to the muddy nature of its water but nevertheless the existence of such a relationship is certainly suggested by the correlations between these features in waters of other regions.

Communities in highly saline lakes

In the four lakes with concentrations above 14,000 p.p.m. the only Entomostraca present were limnetic members of the genera *Artemia*, *Daphnia*, *Moina*, *Diaptomus*, *Cyclops*, and *Cletocamptus*. Both *Diaptomus* and *Cyclops* were represented by two species, the remainder by just one. A typical community in these lakes consisted of *Daphnia longispina*, *Cletocamptus albuquerquensis*, one species of *Diaptomus*, and one of *Cyclops*. It may be noted that all of these components have been taken in lakes of lower salinity and also in fresh water. Aside from them, there were the two exclusively halophil forms *Artemia salina* and *Moina hutchinsoni* which occurred in Little Manitou and Manito Lakes respectively. Thus it is evident that in a range of salinities from approximately 18,000 to slightly over 100,000 p.p.m. the entomostracan fauna is composed mainly of fresh water species with the occasional admixture of truly salt-loving forms. Another noteworthy feature of such communities was that even though few species were present, the number of individuals of some was extremely large. This was particularly evident for *Artemia salina* in Little Manitou, *Diaptomus siciloides* in Big Quill, and *D. tenuicaudatus* in Little Quill and Manito Lakes.

Comparison with Other Areas

The majority of the Entomostraca in these lakes have a distribution that is widespread on this continent and are found in many other parts of the world as well. All of the 31 species of Cladocera occur in other regions of North America and 28 of them have been recorded from Europe and other continental areas. Similarly, among the Copepoda of the families Cyclopidae and Harpacticidae, only *Cletocamptus albuquerquensis* is confined to North America. In the family Centropagidae an entirely different situation exists since it is represented by a single genus *Diaptomus* of which the five species under consideration are restricted to North American waters. On the whole, the distri-

butional data for southern Saskatchewan provide further confirmation of the long-established view of the cosmopolitan nature of plankton organisms in general.

Certain other areas in Canada have a much greater number of entomostracan species besides several genera such as *Latona*, *Holopedium*, *Epischura*, *Heterocope*, and *Eurytemora* which are absent from my collections. Thus Carl (12) on the basis of extensive collections in British Columbia reports 55 species of Cladocera and 33 of Copepoda for that province while Bigelow (8) records 49 species of Cladocera from waters of southwestern Ontario. A large part of this difference may be attributed to sampling of a greater variety of waters in British Columbia and Ontario but it is thought that a crustacean fauna of 31 species of Cladocera and 14 Copepoda in southern Saskatchewan must be considered qualitatively poor. It is not suggested that such differences are the result of saline conditions particularly in view of the occurrence of only 33 species of Cladocera and 19 of Copepoda in 51 lakes of northwestern United States as reported by Kemmerer *et al.* (27).

A similar paucity of species has been noted in the lakes of arid regions situated in other parts of the world. In 17 Siberian lakes having total solids from 215 to 28,325 p.p.m. Decksbach (14) found 18 species of Cladocera and 10 of Copepoda while from a large series of saline waters in Algeria only 9 representatives of each of these groups were reported by Beadle (6). Moreover, 10 of the cladocerans and 3 of the copepods in Siberia and 3 cladocerans and 3 copepods of Algeria were present also in lakes of southern Saskatchewan. It is of interest to note that Beadle found the Cladocera limited to waters of relatively low salinity—only one species occurred in densities above 1.010 (his series ranged from fresh water to about eight times the salinity of sea water).

The comparison may be carried further to include the distribution of species in highly saline waters in other parts of the world. Data for 11 lakes with salinities ranging from about 18,000 to 110,000 p.p.m. (Table VIII) show that the number of species in such concentrations is very small. Aside from the phyllopods which are represented by *Artemia salina* in 110,000 and 68,000 p.p.m. and *Branchinectella salina* in 20,000 p.p.m., there is present usually just one cladoceran along with at least one and sometimes two or three copepods. If attention is confined to identified species it may be seen that the Cladocera are represented by two species of *Daphnia* and three species of *Moina*. The Copepods, on the other hand, comprise three species of *Diaptomus*, three of *Cyclops*, two of *Cletocamptus*, and one of *Echinocamptus*. Thus there appears to be a rather marked uniformity in the Entomostraca of highly saline waters.

Only the phyllopods and the cladoceran *Moina hutchinsoni*, and possibly *Moina microphthalma* and *Echinocamptus viduus*, are found exclusively in high salinities. Also it should be pointed out that *E. viduus* has been recorded so far from Sebkha d'Oran only. All of the remaining Entomostraca have been taken from fresh waters and may be regarded as freshwater species which tolerate highly saline conditions.

TABLE VIII

THE ENTOMOSTRACA IN 11 SELECTED LAKES OF HIGH SALINITY

Lake and locality	Investigator	Approx. salinity	Entomostraca		
			Phyllopoda	Cladocera	Copepoda
Little Manitou (Saskatchewan)	Moore	110,000	*Artemia salina*	*Daphnia longispina*	*Diaptomus siciloides* *Cyclops bicuspidatus* *Cletocamptus albuquerquensis*
Ouargla Chott (Algeria)	Beadle	68,000	*Artemia salina*	—	*Diaptomus salinus* *Cyclops viridis* *Cletocamptus retrogressus*
Winnemucca (Nevada)	Hutchinson	50,000		*Moina hutchinsoni*	*Cletocamptus albuquerquensis*
Mission (North Dakota)	Young	32,500		*Moina macrocopa*	*Cletocamptus albuquerquensis*
Djaman-Ak-Kul (Siberia)	Decksbach	28,000		*Moina microphthalma*	*Diaptomus salinus* *? harpacticid*
Big Quill (Saskatchewan)	Moore	26,000		*Daphnia longispina*	*Diaptomus siciloides* *Cletocamptus albuquerquensis*
Stump (North Dakota)	Young	25,000		*Moina macrocopa*	*Cletocamptus albuquerquensis*
Big Soda (Nevada)	Hutchinson	22,500		*Moina hutchinsoni*	*Cletocamptus albuquerquensis*
Sebka d'Oran (Algeria)	Beadle	20,000	*Branchinectella salina*	*Daphnia atkinsoni*	*Cyclops minutus* *Cletocamptus retrogressus* *Echinocamptus viduus*
Little Quill (Saskatchewan)	Moore	19,000		*Daphnia longispina*	*Diaptomus tenuicaudatus* *Cyclops viridis* *Cletocamptus albuquerquensis*
Manito (Saskatchewan)	Moore	18,000		*Daphnia longispina* *Moina hutchinsoni*	*Diaptomus tenuicaudatus* *Cyclops viridis*

Changes in Distribution in Saline Lakes Over a Period of Years

Very few limnological investigations were made in southern Saskatchewan in the years prior to 1938 but one in particular provided information which may be utilized in a comparison of entomostracan populations and certain environmental factors at dates several years apart (see Tables IX and X). The study in question was that of the Quill Lakes made by Huntsman and Willey in 1920. According to Willey (45) the Entomostraca in Little Quill Lake at that time consisted of *Daphnia longispina*, *Diaptomus sicilis*, *Cyclops viridis*, and *Laophonte calamorum* (in a later publication (47) this was corrected to *L. mohammed* with which it was synonomous). Although most of Willey's material was obtained from Little Quill, his remarks make it quite clear that the same species were present also in Big Quill Lake. Our examinations in 1938 and 1939 showed that Little Quill contained *Daphnia longispina*, *Diaptomus tenuicaudatus*, *Cyclops viridis*, and *Cletocamptus albuquerquensis*, whereas we found *D. longispina*, *Diaptomus siciloides*, and *C. albuquerquensis* in Big Quill Lake.

TABLE IX

ENTOMOSTRACA OF THE QUILL LAKES IN 1920 AND 1938–39. SYMBOLS + AND − INDICATE PRESENCE AND ABSENCE RESPECTIVELY

Species	Little Quill		Big Quill	
	1920	1938–39	1920	1938–39
Daphnia longispina	+	+	+	+
Diaptomus sicilis = *tenuicaudatus*	+	+	+	−
Diaptomus siciloides	−	−	−	+
Cyclops viridis	+	+	+	−
Laophonte mohammed	+	−	+	−
Cletocamptus albuquerquensis	−	+	−	+

TABLE X

ANALYSIS OF QUILL LAKE WATERS IN 1920 AND 1939. COMPOSITION EXPRESSED IN PARTS PER MILLION

Constituents	Little Quill Lake		Big Quill Lake	
	May, 1920*	July 2, 1939	May, 1920*	July 2, 1939
SiO_2	7.4	16.0	10.4	16.0
Ca	180.7	131.0	502.0	117.0
Mg	825.8	1820.0	1169.1	2467.0
Na and K as Na	1872.9	3115.0	2632.7	4511.0
CO_3 and HCO_3 as CO_3	219.0	258.0	235.8	301.0
Cl	1197.5	1435.0	1937.0	2097.0
SO_4	5676.0	11650.0	8368.0	16120.0
Total	9979.3	18425.0	14855.0	25629.0

* *Data from Huntsman (21).*

Contrasting the results of 1938–39 with those of 1920 it is evident that considerable change in the fauna took place. During the interval, *Daphnia longispina* was the only entomostracan which remained common to both lakes. The species of *Diaptomus* determined by Willey as *sicilis* but here called *tenuicaudatus* was still present in Little Quill but absent from Big Quill in which a second form, *Diaptomus siciloides*, has appeared. *Cyclops viridis* was gone from Big Quill in 1938–39 but still occurred in Little Quill. *L. mohammed* disappeared from both lakes and in its place was another harpacticid, *C. albuquerquensis*.

A search for possible causes of these faunal changes points at once to the increases in salt concentration which have taken place since 1920. At that time the salinities of Little Quill and Big Quill were 9979 and 14,855 whereas in 1939 they had risen to 18,425 and 25,629 p.p.m. Such increases would be expected to have pronounced effects on the life in the lakes and it is concluded that they were responsible for the disappearance of *L. mohammed* from Little Quill and of *D. tenuicaudatus*, *C. viridis*, and *L. mohammed* from Big Quill. The increased concentration in each case was largely a result of greater amounts of sulphate and sodium with magnesium also of considerable importance. The high magnesium contents (1820 and 2467 p.p.m.) may have been a limiting factor for *L. mohammed* but apparently not for the other two species, since both were taken in Manito Lake where the magnesium was 3280 p.p.m. Otherwise, there appears to be no indication that this loss of species was occasioned by anything other than the influence of an increase in total salinity acting either directly or indirectly.

There remains the question of additions to the fauna which have occurred at some stage during the time of increasing concentration. It seems reasonable to believe that the presence of *C. albuquerquensis* in Little Quill and of *D. siciloides* and *C. albuquerquensis* in Big Quill can be explained only on the basis of passive dispersal of forms which could live under the existing conditions. Once these animals reached the lakes, they were able to become firmly established as indicated by the abundance of *D. siciloides* in Big Quill Lake.

This description of changes in the Entomostraca of Big Quill lake includes an instance of succession involving two species of the same genus and it may be noted that such cases are not without close parallel in other saline lakes. For example, Young (48) found *Diaptomus sicilis* abundant in Stump Lake in 1912 when the total solids were 19,000 p.p.m., while in 1923 when the concentration was 25,450 p.p.m. this species was absent but *D. siciloides* was abundant.

The inadequacy of data concerning changes in the fauna of other saline lakes in southern Saskatchewan precludes an extensive discussion of this important subject. Since our information suggests that the salt content of many of the lakes has been increasing over a long period of time, there cannot be much doubt but that the life in other highly saline waters has undergone alterations comparable to those already mentioned. The persistence of this condition can be expected to result in further changes, and to affect, as well, other lakes in the area.

Upper Limits of Salinity Tolerance for Entomostraca

As already indicated, some of the Entomostraca in this series of lakes show marked differences in the degrees of salinity which they appear to tolerate. A knowledge of individual cases coupled with similar data from salt waters of other inland areas admits of suggestions as to upper limits for many of these organisms. Correlations with the distribution of widespread forms, particularly those of limnetic habits, provide the most useful information for such an attempt but valuable data are also available for some species which occur in only a small number of lakes. As a rough classification of the species involved we may utilize four categories: (1) species which tolerated salinities above 14,000 p.p.m.; (2) those occurring in concentrations up to 14,000 p.p.m.; (3) those restricted to 3000 p.p.m. and less; and finally, (4) the forms found only in concentrations below 1000 p.p.m. In considering the subject of upper limits, there are two additional observations which must be kept in mind. In the first place, the salinity figures shown above are for one date only and do not represent maxima. Thus in Redberry Lake (concentration given as 13,144 p.p.m.), which appears to be the upper limit for several species, the salt content is known to rise as high as 15,000 p.p.m. and indeed probably surpasses this. Also it is important to note that there are certain significant gaps in this series of salinities, particularly in the higher ranges. No lakes were found with concentrations between 14,000 and 18,348 p.p.m. and only three between 3000 and 11,000 p.p.m. Any or all of the species which have maximum limits at about 14,000 or 3000 p.p.m. might possibly be found in somewhat higher salinities.

Very little can be said about the upper limits for *Daphnia longispina*, *Diaptomus siciloides*, and *Cyclops bicuspidatus* since these occurred even in Little Manitou, the most saline lake in the area. However, all three species were exceedingly rare in this lake and it is suspected that the upper limit for these forms is likely not much beyond the highest known surface concentration of approximately 130,000 p.p.m. *Cletocamptus albuquerquensis* on the other hand was common in Little Manitou Lake and probably has a somewhat higher upper limit than that of the foregoing species. Judging from Beadle's results, the upper limit for *Cyclops viridis* is well above that suggested by our studies (18,688 p.p.m.) and is at least 68,000 p.p.m. (6). The maximum tolerance for *Diaptomus tenuicaudatus* is believed to be somewhere between 20,000 and 25,000 p.p.m.

The widespread forms, *Ceriodaphnia quadrangula*, *Diaphanosoma*, *leuchtenbergianum*, *Daphnia pulex*, *Bosmina obtusirostris*, *Chydorus sphaericus*, *Pleuroxus denticulatus*, *Cyclops serrulatus*, and the geographically restricted species *Scapholeberis mucronata* and *Diaptomus shoshone*, were confined to salinities below 14,000 p.p.m. None of these has been reported from higher concentrations in other localities. It is suggested that a probable upper limit for them would be between 15,000 and 16,000 p.p.m. From distributions in saline lakes elsewhere it appears that *Daphnia magna*, *Simocephalus vetulus*, and *Alona rectangula* should be included also in this group. *Laophonte mohammed*

in southern Saskatchewan was limited to salinities below 14,000 p.p.m. but from Beadle's record it obviously has an upper limit much higher than this—possibly at least 60,000 p.p.m. The occurrence of *C. viridis* and the four last mentioned species in higher concentrations in other regions serves to emphasize the possibility that factors other than salinity are involved in determining distributions.

Ceriodaphnia lacustris, *Alona costata*, *Leptodora kindtii*, *Cyclops leuckarti*, and *Cyclops albidus* were widely occurring forms, while *Alona affinis*, *Pleuroxus aduncus*, *Alona guttata*, *Leydigia quadrangularis*, *Camptocercus rectirostris*, *Graptoleberis testudinaria*, *Eurycercus lamellatus*, *Macrothrix laticornis*, *Diaptomus oregonensis*, *Cyclops fimbriatus*, and *Canthocamptus dentatus* were species of limited distribution, all of which were taken in salinities of 3000 p.p.m. and less. A tentative upper limit for the members of this group, excluding *Cyclops leuckarti* and *Cyclops fimbriatus*, is set at between 3000 and 4000 p.p.m. The two exceptions have been recorded, in other areas, from concentrations which indicate upper limits in the neighborhood of 23,000 and 8500 p.p.m. respectively. Several of the other species in this group were present in just a small number of lakes and the suggested limit may well be too low for some of them.

Sida crystallina, *Chydorus gibbus*, *Polyphemus pediculus*, *Bosmina longispina*, *Acroperus harpae*, *Ceriodaphnia reticulata*, *Ilyocryptus acutifrons*, *Alonella nana*, and *Diaptomus ashlandi* were also species with occurrence limited to very few lakes all of which, in this case, had salinities below 1000 p.p.m. The last three species may be entirely restricted to fresh or slightly saline conditions but in any case the tolerance limit for the whole group is not considered to be much higher than 1000 p.p.m.

Adaptation to Saline Conditions

It has been indicated above that salinity often varies to a considerable extent from lake to lake and in some cases undergoes fairly wide seasonal changes. Also it has been shown that the various species of freshwater Entomostraca differ in their apparent ability to withstand saline environments. Such a result is not unexpected since existence under these circumstances obviously entails adaptation to a greater range of conditions than may be considered normal for such forms and all will not show the same degree of adaptive ability. Furthermore, all freshwater animals are believed to have body fluid concentrations which are hypertonic to the customary external medium and it seems likely that they will be restricted to salinities which correspond to the levels of blood concentration. We have no experimental data regarding the latter point but our distributional records suggest that the internal concentrations must vary considerably among the Entomostraca. That such variations do exist for freshwater animals in general is shown by the observations of several investigators as summarized by Beadle and Cragg (7, p. 159), and the same authors conclude that "most fresh-water animals have a low blood concentration." Also from the work of Fritsche (16) with *Daphnia magna* and of Beadle and Cragg (7) with *Gammarus pulex* it is evident

that the internal concentration of these freshwater Crustacea can change to some extent to correspond to alterations in the external medium. Taking into consideration all sources of information, there seems to be justification for assuming that differences in blood concentrations as well as some measure of acclimation apply to all freshwater Entomostraca and that both normal and maximum values are relatively low in most cases. Salinity thus can act directly as the controlling factor in the occurrence of Entomostraca by eliminating the various species at levels corresponding to the maximum body fluid concentrations which can be tolerated.

A total of 38 of the 44 species of Cladocera and Copepoda occurred in lakes having salinities of 14,000 p.p.m. or less and the foregoing explanation seems adequate to account for the observed distributions. The majority and perhaps all of these species can withstand some degree of salinity but apparently none is able to maintain the internal concentration at sublethal levels under highly saline conditions. On the basis of all data from this study, it is suggested that the maximum external salinity that can be tolerated by most freshwater animals is probably between 4000 and 15,000 or possibly 16,000 p.p.m. This view conforms quite closely to that of Beadle (6) who concludes that the tentative limits are equivalent to about 20 to 40% sea water (density 1.004–1.009).

In contrast to the main group discussed above, there were six species, *Daphnia longispina*, *Diaptomus siciloides*, *Diaptomus tenuicaudatus*, *Cyclops bicuspidatus*, *Cyclops viridis*, and *Cletocamptus albuquerquensis*, which ranged above what may be regarded as the upper limit for most freshwater forms. All of these animals have been collected in low salinities and also in fresh water, hence there seems to be no a priori reason for believing that they have normal internal concentrations which are unusually high. However, it is conceivable that especially salt tolerant races may have developed in the more saline lakes. Such a consideration may well explain the presence of *D. tenuicaudatus* in Manito and Little Quill Lakes where salinities are in the neighborhood of 20,000 p.p.m. The other species, however, tolerate much higher salinities and for them it seems necessary to assume the existence of some mechanism, with efficiency varying according to the species, which is able to maintain hypotonic body fluids in high salt concentrations. If this assumption is true, then it is obvious that for these species the body fluids must be hypertonic to part of their salinity range and hypotonic to the rest. Beadle (5) has shown that a situation of this nature can occur under experimental conditions in the larva of the saline water mosquito, *Aedes detritus*.

There remain finally the strictly halophil forms, *Artemia salina* and *Moina hutchinsoni*. The former maintains its internal concentration at a level which is characteristic of the range shown by freshwater animals (7, 33). Judging from the concentration of natural habitats it is quite evident that this species possesses a powerful mechanism for maintaining body fluids hypotonic to the surrounding medium. Present information concerning *Moina hutchinsoni* supports the belief that a similar set of conditions apply to it.

Indirect Effects of Salinity on Distribution

Salinity may be of importance also in that it has an effect on certain factors of a biological nature which undoubtedly condition the occurrence of Entomostraca. One of the factors that is considered to be definitely influenced by salt concentration is the potential food supply for these organisms. Although no detailed study of the food of Entomostraca was attempted in this investigation, it is evident from the work of Naumann (35 and 36), Klugh (28), and others that for most of these forms it consists mainly of bacteria, detritus, and planktonic Chlorophyceae. Birge (9) mentions that the predaceous forms *Polyphemus pediculus* and *Leptodora kindtii* subsist on protozoans, rotifers, and minute crustaceans. No information is available regarding the bacterial and protozoan components but the marked restrictions of green algae and rotifers in the more saline lakes may be quite significant (38, 29). A similar limitation of Chlorophycae in a large series of lakes in North Dakota has been reported by Moore and Carter (34) who state that "Many of the species seem to be very intolerant of increasing concentration of salts, and are therefore eliminated from the flora of the strongly alkaline lakes." It is assumed then that the indirect effect of salinity on available food materials plays some part in the distribution of Entomostraca in this area. It is quite possible that *Cyclops viridis*, for example, has disappeared from Big Quill Lake because of changes in food accompanying increasing salinity and the same may apply to *Laophonte mohammed* which has vanished from both of the Quill Lakes.

A second indirect effect of salinity concerns its possible influence on the habitats available for littoral species in these lakes. Considering the eight lakes which were the subject of special studies in 1940 it is evident that both the number of littoral species of Entomostraca and the number of species of rooted aquatics were greatest in lakes of low salt concentration and showed a decrease with increasing salinity (see Table XI). Thus, Turtle Lake (370 p.p.m.) contained 16 species of Entomostraca and 10 species of rooted plants in the littoral region, whereas in Little Manitou Lake (106,693 p.p.m,) both

TABLE XI

SALINITIES, NUMBER OF ROOTED AQUATICS, AND NUMBER OF LITTORAL SPECIES OF ENTOMOSTRACA IN EIGHT LAKES

Lake	Salinity, p.p.m.	Number of species of rooted aquatics	Number of littoral species of Entomostraca
Turtle	370	10	16
Murray	864	8	12
Jackfish	1388	5	6
Echo	1407	5	5
Last Mountain	2653	5	5
Stoney	7167	2	1
Redberry	13,144	2	4
Little Manitou	106,693	0	0

littoral Entomostraca and rooted aquatics were absent. The available data suggest that similar considerations apply to all of the lakes in the series. It is admitted that the existence of such correlations is not proof of these relationships but, nevertheless, seems strongly suggestive of them. The assumption is that increasing salinity brings about a reduction in the number of ecological niches available for littoral species and that these accordingly disappear. As far as the writer is aware, very little if anything, is known about associations between various species of Entomostraca and different types of aquatic plants. This subject would seem to be a promising field for the investigation of the effect of different types of cover on presence or absence of planktonic Crustacea.

It is significant that no littoral species of Entomostraca were found in lakes with salinities above 14,000 p.p.m. This absence may be a result of unadaptability to such conditions or it is equally likely that it is a result of limitations in food supply or lack of proper habitats, perhaps both, in these lakes. In any case, it is believed that salinity is the basic factor and that the others vary with it.

Factors Involved in Geographic Distribution

One of the primary considerations in a study of geographic distribution is adaptability to environmental conditions. Every organism of inland aquatic habitats must adapt to a whole complex of physical, chemical, and biological influences. Owing to the intricate nature of actions and interactions, it is difficult to assess the relationships of individual factors to the presence or absence of flora and fauna. In the foregoing sections it has been assumed that the degree of adaptability to saline conditions is the dominant feature governing the distribution of Entomostraca in lakes of southern Saskatchewan. While the data seem to warrant this assumption, it is necessary to consider the importance of other phenomena as agents controlling distribution.

Detailed studies of freshwater environments have often resulted in the naming of certain factors as dominant in the determination of geographic distribution of special organisms or groups of organisms. Thus temperature is believed by Marsh (30) to be the chief factor controlling the distribution of Copepoda. Carl (12) states also that temperature is of considerable significance to the presence or absence of both Copepoda and Cladocera in lakes of British Columbia. Our data contain no indication that temperature plays any noticeable part in the distribution of Entomostraca in the saline lakes of Saskatchewan. It may have been of importance in determining what species could become established in this area since temperature variations of 0 to 20° C. would favor eurythermal forms.

As already mentioned, there may be a relationship between size of lake and distribution of *Leptodora kindtii*, and between argillotrophic conditions of Antelope Lake and its peculiar entomostracan association. These appear to be the only indications that physical and chemical conditions, other than salinity, are important in governing the distribution of Entomostraca in southern Saskatchewan.

Every species is believed to occupy a definite place or niche within a community. Since closely related forms are undoubtedly competitors for the occupation of the same niche, they are rarely found together in a major habitat within a lake. Also, since plankton organisms as a whole tolerate such a wide range of physical and chemical conditions, it seems likely that once a species becomes established, it will not be replaced unless a related form which is more adaptable reaches the lake. Interspecific relationships, involving the mutual exclusiveness of closely related species, may well be one of the most significant factors in determining geographic distributions. Perhaps this furnishes an explanation of the discontinuous occurrence of forms like *Ceriodaphnia quadrangula* and *C. lacustris*, the species of *Diaptomus* (excluding *D. shoshone*), and others.

Closely linked with adaptability to environmental conditions is the matter of the means of dispersal available to Entomostraca—a subject which has been of interest to many investigators of plankton distribution. The production of winter or "resting" eggs is apparently a phenomenon of general occurrence among the Cladocera (44) and is known also for some species of *Diaptomus* (18). Resting eggs are unknown in *Cyclops* but it is suggested by Gurney (18) that most species of this genus possess some means of surviving unfavorable conditions (e.g. aestivation of copepodid stages). Such dormant stages are particularly amenable to wide dissemination by water, birds, and winds. Also, summer eggs as well as immature forms and adult animals could in all likelihood be carried from place to place by flowing water and by birds. Thus the potentialities for the passive dispersal of Entomostraca over extensive areas are very great. There is, in addition, the possibility of active dispersal but it is a method which is probably of much less importance to animals of this type.

The distribution of two species of *Diaptomus* in southern Saskatchewan shows evidence of dispersal by drainage systems. Thus *D. siciloides* was found in Last Mountain, Pasqua, Echo, and Round Lakes, all of which belong to the Qu'Appelle River drainage. The same species was taken in Fishing and Margo Lakes which were connected in former years. Similarly Murray and Jackfish Lakes, which are united by a permanent stream, contained *D. tenuicaudatus* as did Midnight and Helene Lakes which are joined at high water. The existence of the same species in lakes which are connected now, or have been in the past, suggests the importance of water transport as a factor of dispersal. But the presence of different species of *Diaptomus* in Kenosee and Carlyle Lakes, which are also united at high water, indicates that distributions are determined by other conditions as well.

The marked uniformity of the whole crustacean zooplankton in some connected lakes may also provide evidence relating to the importance of dispersal by water transport. As an example, 14 of the 23 species in Murray Lake occurred in Jackfish Lake which had a total of 16 species. The similarity is further accentuated by the consideration that three of the species in Murray Lake could be absent from Jackfish because of salinity differences (864 and

1388 p.p.m.). Also, 12 of the 13 species in Pasqua Lake were found in Echo Lake which is connected with it during at least part of each summer. This evidence is perhaps partly nullified by the fact that only six species were common to the four lakes of the Qu'Appelle drainage and six or seven to the other pairs of lakes mentioned above. At the same time, it must be recognized that conditions in certain portions of drainage areas may prove more suitable to some species than to others and that even though dispersal by water transport does take place, the degree of adjustment to the new environment may not be adequate for survival.

Totally isolated lakes in many cases showed populations which were similar in six or seven and sometimes more components. The general resemblance of plankton communities throughout this series of lakes perhaps suggests that interconnected lakes were at one time the rule rather than the exception. This situation could have been true in an area as flat as southern Saskatchewan and with the greater amounts of water that undoubtedly existed in past years. From all the evidence at hand it would appear that dispersal by water transport may have been a chief factor influencing distributions in this area.

There are also instances of the effect of dispersal by other means. Certainly the appearance of *Diaptomus siciloides* and *Cletocamptus albuquerquensis* in Big Quill Lake and of *C. albuquerquensis* in Little Quill Lake cannot be ascribed to water transport. The agency of birds or of wind, more likely the former, must have been involved. In all probability *C. albuquerquensis* was transferred to the Quill Lakes from nearby Little Manitou Lake or from the Devil's Lake region of North Dakota which lies directly to the south. *D. siciloides* could have been introduced from several lakes to the south and to the east of Big Quill Lake.

Origin of the Fauna

The origin of the present entomostracan populations goes back to the end of glacial times for it seems highly probable that when the Keewatin icesheet covered the area under consideration, there was an obliteration of these organisms along with all other flora and fauna. Following the retreat of ice, this central region may have been repopulated from all directions but most forms could be expected to come in from the south and southwest. For the majority of the species represented, geographic distributions are so widespread that there are no indications of specific points of origin. Among the species of *Diaptomus* there appears to be evidence of dispersal from the south and west for *D. shoshone* and from the south for *D. ashlandi*, *D. oregonensis*, and *D. siciloides*. The first occurs in mountain lakes of the western United States and southern Canada while the remaining species are common across the northern part of the United States with *D. siciloides* being found farthest to the south. Data from southern Saskatchewan include the most northerly records for *D. oregonensis* and *D. siciloides* and, apart from Young's reports in North Dakota, the most easterly for *D. shoshone*. The distribution of *D. tenuicaudatus* suggests that its center of origin may be within the area under

consideration. Another species for which a southern point of origin appears indicated is *Cletocamptus albuquerquensis* which has been reported from New Mexico north to central Saskatchewan. It also seems quite possible that the same may be true of the recently discovered cladoceran, *Moina hutchinsoni.*

Summary and Conclusions

1. The present study provides data on the distribution of 49 species of Entomostraca in 48 lakes in southern Saskatchewan. It is based on materials collected in the course of a limnological survey carried on during the summers of 1938 to 1941.

2. The location of the lakes is shown in a map, Fig. 1. Physical and chemical observations are presented in Table I. Areas ranged from 0.12 to 162 sq. miles, the majority being 5 sq. miles or less. Most lakes are comparatively shallow (maximum depths of 30 ft. or less) and uniformly warm from surface to bottom. Only a few of the deepest lakes show thermal stratification. The quantities of dissolved oxygen were high in nearly all lakes. In a small number there was evidence of extreme bottom stagnation. With few exceptions the pH ranged from 8.3 to 8.9. Light penetration as measured by Secchi's disk was usually between 7 and 17 ft.

3. There are few regions which offer such extensive possibilities for study of inland saline waters as the southern half of the Province of Saskatchewan. Here, under the influence of climatic and physiographic factors, most lakes have developed definitely saline conditions. Detailed mineral analyses for 41 lakes are given in Table II and total residue determinations for the remaining seven in Table III. The lakes form a series ranging from fresh to extremely saline waters. The salinity of 20 lakes lies between 200 and 1000 p.p.m.; of 20 others it is from 1000 to 10,000 p.p.m.; of 7 between 10,000 and 30,000 p.p.m. Little Manitou Lake with more than 100,000 p.p.m. is an example of extreme salinity.

4. Lakes in the prairie and parkland show well-developed saline conditions while those of the forest area are generally fresh. The composition of the waters indicates that the former are predominantly sulphate while the latter are carbonate types. The salinity of the prairie and parkland lakes has been increasing for many years. There are also seasonal changes in the salt content in these waters. During the summer months the concentration often increases by as much as 5 to 8% from the spring level.

5. Some species of Entomostraca were widespread while others were collected in only a small number of lakes and a few in just one locality. Most species with a limited geographic distribution are characteristic of the littoral regions and insufficient sampling of such habitats may be a factor in the apparent restriction in their distribution.

6. *Artemia salina* was found only in Little Manitou Lake. The chemical composition of natural environments of this species is discussed and considered

to consist of highly saline waters which contain high sodium and chloride and low potassium concentrations with other constituents being presumably unimportant.

7. Our data suggest that, of the physical and chemical factors, salinity is most important in controlling the distribution of Entomostraca in southern Saskatchewan. Records of the presence of Cladocera and Copepoda in the different lakes are presented in Table IV. The effect of different degrees of salinity in determining the distribution of these forms is considered a result of the total concentration rather than the relative amounts of individual ions.

8. There are pronounced differences in the degree of salt concentration in which the various species are found. On the basis of salinity ranges there appear to be five main groups among the Entomostraca: (*a*) species which tolerate salinities up to 1000 p.p.m.—some exclusively freshwater forms may be included here; (*b*) species capable of withstanding concentrations up to 4000 p.p.m.; (*c*) those which occur in salinities up to 15,000 p.p.m.; (*d*) forms which tolerate high salt contents; and (*e*) two exclusively halophil species.

9. The number of species of Cladocera and Copepoda decreases as salinity increases. Lakes with salinities less than 1000 p.p.m. may have up to 26 species while in 15,000 p.p.m. only half this number are present. Further reductions occur in still higher salinities until in 100,000 p.p.m. only four species are left. The Cladocera are much more severely restricted by high salinity than are the Copepoda. Only 2 of the 31 species of Cladocera are found in salinities above 15,000 p.p.m. whereas there are 5 of the 14 Copepoda of which this is true.

10. Littoral species are apparently more susceptible to increasing concentration than are those of limnetic habits. In salinities above 15,000 p.p.m. littoral species were absent.

11. With the exception of *Artemia salina* and *Moina hutchinsoni*, the crustacean component of the zooplankton in these lakes consists of typically freshwater species. Most of these forms are widely distributed in North America and many of them are found also in other parts of the world. Only nine species are confined to North America.

12. In highly saline waters a genus was never represented by more than one species. The same was true in general for salinities below 15,000 p.p.m. but there were some exceptions notably in *Daphnia*, *Diaptomus*, and *Cyclops*. The occupation of different niches is believed to be an explanation of the association of closely related species in the same lake and in the same major habitat of a lake. Typical entomostracan communities in these lakes are discussed.

13. The saline lakes of the area contain far fewer species than do the lakes in other parts of Canada such as British Columbia and Ontario. However, the number of forms is somewhat greater than those recorded from similar series of waters in Algeria and Siberia.

14. With the exception of the phyllopods, there is a remarkable uniformity in the entomostracan population of highly saline waters in various parts of the

world. Data for 11 lakes with salinities between 18,000 and 110,000 are given in Table VIII. The components consist usually of one cladoceran and at least one and sometimes two or three copepods. Both halophil and freshwater species are regularly represented in lakes of high concentrations with the latter being in the majority.

15. Changes in the Entomostraca of the Quill Lakes over a period of 20 years are shown in Table IX. Some species have disappeared while others have appeared in these lakes. The losses are considered to have been a direct result of increasing salt concentration. Additions are attributed to passive dispersal and subsequent establishment of more salt tolerant forms. Further increases in the salt concentrations of this series of lakes may be expected to result in marked alterations in the fauna.

16. Data from this study and from investigations of saline waters in other areas are utilized in proposing tentative upper limits of salinity tolerance for many of the species under consideration. The upper limit for most freshwater organisms is probably between 4000 and 16,000 p.p.m.

17. The physiology of adaptation to saline conditions is discussed briefly. Salinity may bring about its effect directly, through osmotic relations, or indirectly, through its influence over available food, and habitats for littoral species. It is suggested that the Entomostraca may be divided into two groups on the basis of osmoregulatory ability: (*a*) those which have a powerful mechanism for maintaining hypotonic body fluids in highly saline waters and (*b*) those which have no such mechanism. Members of the latter group, which includes most of the species, are limited by the maximum body fluid concentration which can be tolerated. While this varies with the species, it is apparently low in most instances.

18. Interspecific relationships and means of dispersal are considered secondary to salinity as factors governing distribution in these lakes.

19. The entomostracan fauna of southern Saskatchewan consists in general of species which have a widespread geographic distribution and undoubtedly it has been derived from many sources. Definite points of origin seem indicated for only a few species of Copepoda.

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References

1. AMERICAN PUBLIC HEALTH ASSOCIATION. Standard methods for the examination of water and sewage. 8th ed. Am. Pub. Health Assoc., New York. 1936.
2. ANNANDALE, M. The fauna of brackish ponds at Port Canning, Lower Bengal. Records Indian Museum, 1 : 35–43. 1907.
3. BAAS-BECKING, L. G. M. Salt effects on swarmers of *Dunaliella viridis* Teod. J. Gen. Physiol. 14 : 765–779. 1931.
4. BAJKOV, A. Report on Manitou Lake, Saskatchewan. Biological Board Can. Manuscript. 1927.
5. BEADLE, L. C. Regulation of the haemolymph in the saline water mosquito larva *Aedes detritus* Edw. J. Exptl. Biol. 16 : 346–362. 1939.
6. BEADLE, L. C. An ecological survey of some inland saline waters of Algeria. J. Linnean Soc. London, Zool. 41 : 218–242. 1943.
7. BEADLE, L. C. and CRAGG, J. B. Studies on adaptation to salinity in *Gammarus* spp. 1. Regulation of blood and tissues and the problem of adaptation to fresh water. J. Exptl. Biol. 17 : 153–163. 1940.
8. BIGELOW, N. K. Representative Cladocera of south-western Ontario. Univ. Toronto Studies Biol. Ser. No. 20 : 111–125. 1922.
9. BIRGE, E. A. The water fleas (Cladocera). *In* Fresh-water biology, *edited by* Ward and Whipple. John Wiley & Sons, Inc., New York. 1918. pp. 676–740.
10. BOONE, E. and BAAS-BECKING, L. G. M. Salt effects on eggs and nauplii of *Artemia salina* L. J. Gen. Physiol. 14 : 753–763. 1931.
11. BREHM, V. Zwei neue Moina-Formen aus Nevada, U.S.A. Zool. Anz. 117 : 91–96. 1937.
12. CARL, G. C. The distribution of some Cladocera and free-living Copepoda in British Columbia. Ecol. Monographs, 10 : 55–110. 1940.
13. CLARKE, F. W. The data of geochemistry. 5th ed. U.S. Geol. Survey Bull. 770. Washington. 1924.
14. DECKSBACH, N. K. Seen und Flüsse des Turgai-Gebietes (Kirgisen-Steppen). Verhandl. Int. Ver. Limnol. 2 : 252–288. 1924.
15. FLORENTIN, R. Faune des mares salées. Ann. sci. nat. Zool. 10 : 209–346. 1899.
16. FRITSCHE, H. Studien über die Schwankungen des osmotischen Druckes der Körperflüssigkeiten bei *Daphnia magna*. Intern. Rev. ges. Hydrobiol. Hydrog. 8 : 22–80, 125–203. 1916.
17. GAUTHIER, E. F. et GAUTHIER, H. Recherches sur la Faune des Eaux Continentales de l'Algérie et de la Tunisie. Imp. Minerva, Alger. 1928. (From L. C. Beadle, 1943.)
18. GURNEY, R. British fresh-water Copepoda. Vols. 1, 2, and 3. London. 1931–33.
19. HERRICK, C. L. and TURNER, C. H. Synopsis of the Entomostraca of Minnesota. 2nd Rept. State Geologist, Geol. Nat. Hist. Survey Minn., Zool. Ser. II. 1895.
20. HESSE, R., ALLEE, W. C., and SCHMIDT, K. P. Ecological animal geography. John Wiley & Sons, Inc., New York. 1937.
21. HUNTSMAN, A. G. The Quill Lakes of Saskatchewan and their fishery possibilities. Contribs. Can. Biol. (n.s.) 1 : 127–141. 1922.
22. HUTCHINSON, G. E. Experimental studies in ecology. 1. The magnesium tolerance of Daphniidae and its ecological significance. Intern. Rev. ges. Hydrobiol. Hydrog. 28 : 90–108. 1933.
23. HUTCHINSON, G. E. A contribution to the limnology of arid regions. Trans. Conn. Acad. Arts Sci. 33 : 47–132. 1937.
24. HUTCHINSON, G. E., PICKFORD, G. E., and SCHUURMAN, J. F. M. A contribution to the hydrobiology of pans and other inland waters of South Africa. Arch. Hydrobiol. 24 : 1–154. 1932.
25. IDE, F. P. Mayflies of two tropical genera, *Lachlania* and *Campsurus*, from Canada with descriptions. Can. Entomologist, 73 : 153–156. 1941.
26. JUDAY, C. Limnological apparatus. Trans. Wisconsin Acad. Sci. 18 : 566–592. 1916.
27. KEMMERER, G., BOVARD, J. F., and BOORMAN, W. R. Northwestern lakes of the United States: biological and chemical studies with reference to possibilities in production of fish. Bull. U.S. Bur. Fisheries, 39 : 51–140. 1923.
28. KLUGH, A. B. The ecology, food-relations and culture of fresh-water Entomostraca. Trans. Roy. Can. Inst. 16 : 15–98. 1927.

29. KUEHNE, P. E. The phytoplankton of southern and central Saskatchewan. Parts I and II. Can. J. Research, C, 19 : 292–322. 1941.

30. MARSH, C. D. Copepoda. *In* Fresh-Water biology, *edited by* Ward and Whipple. John Wiley & Sons, Inc., New York. 1918. pp. 741–789.

31. MARSH, C. D. Distribution and key of the North American copepods of the genus *Diaptomus*, with the description of a new species. No. 2785. Proc. U.S. Natl. Museum, 75 : 1–27. 1929.

32. MARTIN, E. G. and WILBUR, B. C. Salt antagonism in Artemia. Am. J. Physiol. 55 : 290–291. 1921.

33. MEDWEDEWA, N. B. Ueber den osmotischen Druck der Hämolymphe von *Artemia salina*. Z. vergleich. Physiol. 5 : 547–554. 1927.

34. MOORE, G. T. and CARTER, N. Algae from lakes of the northeastern part of North Dakota Ann. Missouri Botan. Garden, 10 : 393–422. 1923.

35. NAUMANN, E. Über die natürliche Nahrungen des limnischen Zooplanktons. Ein Beitrag zur Kenntnis des Stoffhaushalt im Süsswasser. Lunds Univ. Årsskr. II, 14 : 1–48. 1918. (From a summary by P. S. Welch, 1952.)

36. NAUMANN, E. Spezielle Untersuchungen über die Ernährungsbiologie des Tierischen Limnoplanktons. II. Über den Nahrungswerb und die natürliche Nahrung der Copepoden und der Rotiferen des Limnoplanktons. Lunds Univ. Årsskr. II, 19 : 3–17. 1923. (From a summary by P. S. Welch, 1952.)

37. PEARSE, A. S. The fairy shrimps (Phyllopoda). *In* Fresh-water biology, *edited by* Ward and Whipple. John Wiley & Sons, Inc., New York. 1918. pp. 661–675.

38. RAWSON, D. S. and MOORE, J. E. The saline lakes of Saskatchewan. Can. J. Research, D, 22 : 141–201. 1944.

39. RUSSELL, I. C. Geological history of Lake Lahontan. Monographs U.S. Geol. Survey, 11. 1885.

40. SCHÄFER, H. W. Unsere Kenntnis der Copepodenfauna des Brackwassers, nebst Notizen über die Brackwasserfauna von Hiddensee. Intern. Rev. ges. Hydrobiol. Hydrog. 29 : 265–273. 1933.

41. SEWELL, R. B. S. A study of the fauna of the salt lakes, Calcutta. Records Indian Museum, 36 : 45–121. 1934.

42. WALKER, E. M. A preliminary list of the Odonata of Saskatchewan. Can. Entomologist, 72 : 26–35. 1940.

43. WELCH, P. S. Limnology. 2nd ed. McGraw-Hill Book Company, Inc., New York and London. 1952.

44. WELDON, W. F. R. Crustacea. *In* Cambridge natural history, *edtied by* Harmer and Shipley. Chap. 2. Vol. 4. Macmillan and Co. Limited, London. 1909. pp. 18–54.

45. WILLEY, A. Notes on the distribution of free-living Copepoda in Canadian Waters. Contribs. Can Biol. (n.s.) 1 : 305–334. 1923.

46. WILLEY, A. Ecology and the partition of biology. Trans. Roy. Soc. Can., V, 17 (3) : 1–9. 1923.

47. WILLEY, A. Copepod phenology.—Observations based on new material from Canada and Bermuda. Arch. Zool. Ital. 16 : 601–617. 1931.

48. YOUNG, R. T. The life of Devils Lake, North Dakota. Pub. North Dakota Biol. Sta. 1924.

Appendix

TABLE XII

COMPOSITIONS OF 41 LAKES EXPRESSED AS PERCENTAGES OF SALINITIES

Lake	Salinity	SiO_2	Fe_2O_3 Al_2O_3	Ca	Mg	K	Na	CO_3	HCO_3	Cl	SO_4	Location
Little Manitou	106693	—	0.2	0.5	10.4	1.0	16.8	0.2	0.5	21.8	48.4	Prairie
Big Quill	25911	—	0.2	0.5	9.5	—	17.4	0.3	1.7	8.2	62.0	Parkland
Little Quill	18668	0.1	0.3	0.7	9.8	—	16.7	0.4	2.0	7.7	62.5	Parkland
Manito	18348	0.3	0.1	0.4	17.9	—	4.5	5.4	9.8	8.5	53.1	Parkland
Bitter	13180	0.1	—	0.1	9.1	—	18.0	1.8	5.1	1.9	64.0	Forest
Redberry	13144	—	0.1	0.5	12.1	0.8	11.8	0.7	3.7	1.1	69.0	Parkland
Antelope	12803	0.2	0.2	0.2	8.8	—	18.2	1.8	5.6	2.5	62.4	Prairie
Basin	11398	0.1	0.1	1.4	11.5	—	12.7	0.8	4.5	3.8	65.2	Parkland
Stoney	7167	0.2	0.2	1.7	14.4	2.5	5.7	0.5	4.1	0.9	70.0	Parkland
Lenore	5313	0.4	0.1	0.9	12.8	—	10.8	2.0	7.7	2.9	62.2	Parkland
Fishing	3011	0.5	0.1	2.8	12.3	—	9.3	1.3	9.3	1.7	62.8	Parkland
Wakaw	2885	0.1	—	6.7	10.0	—	8.7	0.1	7.0	1.9	65.3	Parkland
Last Mountain	2653	0.5	0.2	1.7	6.3	0.6	19.8	1.3	13.8	6.7	49.0	Prairie
Atten's	1672	0.4	0.1	0.4	7.2	—	18.5	4.4	40.5	1.9	26.6	Parkland
Carlyle	1470	0.6	0.1	2.4	14.4	—	5.7	4.9	28.0	1.3	42.7	Prairie
Margo	1440	0.7	0.2	6.0	6.1	—	14.8	—	14.0	0.5	57.7	Parkland
Echo	1407	0.7	0.2	4.3	5.0	1.6	18.0	1.1	19.5	7.5	42.3	Parkland
Jackfish	1388	0.6	0.1	1.6	10.2	2.0	11.5	3.3	27.8	1.0	41.8	Parkland
Round (P.A.)	1267	0.5	0.1	3.7	14.4	—	3.8	0.6	18.2	0.2	58.4	Forest
Kenosee	1252	0.8	0.2	3.3	14.7	—	3.0	5.0	24.7	1.5	46.8	Prairie
Kenderdine	1237	0.3	0.1	1.7	6.3	—	18.0	3.0	41.7	0.8	28.0	Parkland
Pasqua	1092	2.0	0.3	5.3	5.0	—	16.9	0.8	24.6	6.0	39.1	Parkland
Wilson	1067	1.8	0.1	4.5	16.4	—	1.5	17.1	33.6	0.6	24.3	Parkland
Clearwater	1027	1.1	0.2	0.9	13.2	—	8.2	8.7	52.6	2.0	13.0	Prairie
Round (Qu'App.)	1009	1.8	0.2	5.2	5.3	—	16.8	1.4	24.3	5.8	39.2	Parkland
Good Spirit	952	4.1	0.1	4.3	13.4	—	4.1	4.3	24.8	0.8	44.1	Parkland
Edward's	923	0.9	0.4	5.5	12.0	—	5.3	1.1	24.5	0.4	50.0	Parkland
Murray	864	0.8	0.1	4.2	9.6	0.9	10.4	3.2	28.1	1.0	41.8	Parkland
Birch	803	3.8	0.1	1.1	13.8	—	5.5	9.7	63.6	0.2	2.3	Forest
Christopher	695	0.5	0.1	2.2	13.7	—	4.7	4.7	59.4	0.2	14.6	Forest
Emma	695	1.0	0.1	2.7	12.8	—	5.2	4.4	57.2	0.3	16.3	Forest
Brightsand	634	2.2	0.2	1.8	12.7	—	6.0	6.2	66.5	0.3	4.1	Forest
Pelletier	625	1.4	0.1	3.7	10.9	—	8.0	6.2	52.2	1.4	16.0	Prairie
Sturgeon	576	2.0	0.2	11.0	7.7	—	4.9	3.0	40.6	0.3	30.2	Forest
Little Loon	502	1.2	0.1	4.7	12.0	—	3.7	4.8	70.0	0.3	3.3	Forest
Greenwater	491	3.6	0.5	9.8	7.2	—	7.1	2.1	35.4	4.4	29.7	Forest
Madge	449	4.0	0.2	11.0	10.1	—	1.1	2.8	32.5	0.4	37.8	Forest
Deep	418	1.3	0.1	10.1	8.1	—	3.4	2.9	64.8	0.1	9.3	Forest
Turtle	370	2.4	0.3	6.5	8.1	—	7.4	2.5	68.6	0.5	3.8	Forest
Meeting	315	0.9	0.5	7.2	10.0	—	5.2	5.2	62.4	—	8.6	Forest
Helene	297	0.6	0.3	6.8	9.5	—	4.2	0.4	76.8	0.6	0.7	Forest

CONTENTS OF VOLUME 30

ANGUS, T. A.—The aerobic bacteria associated with the eastern hemlock looper, *Lambdina fiscellaria* (Gn.), 208.

ARNOLD, J. W.—Effects of certain fumigants on haemocytes of the Mediterranean flour moth, *Ephestia kuhniella* Zell. (Lepidoptera: Pyralididae), 365. The haemocytes of the Mediterranean flour moth, *Esphestia kühniella* Zell. (Lepidoptera: Pyralididae), 352.

AUCLAIR, J. L.—See Maltais, J. B.

AUCLAIR, J. L. and DUBREUIL, R.—A simple ultramicromethod for the quantitative estimation of amino acids by paper partition chromatography, 109.

BLAIS, J. R.—The relationship of the spruce budworm (*Choristoneura fumiferana*, Clem.) to the flowering condition of balsam fir (*Abies balsamea* (L.) Mill.), 1.

BONDAR, G. F.—See McLeod, J. A.

BRENNAN, J. M.—See Brown, J. H.

BRIDGEFORD, H. B. and PELLUET, D.—Induced changes in the cells of the ovotestis of the slug, *Deroceras reticulatum* (Muller), with special reference to the nucleolus, 323.

BROWN, J. H. and BRENNAN, J. M.—A note on the chiggers (Trombiculidae) of Alberta, 338.

BRYGIDER, W.—In what embryonic stage do the eggs of *Neodiprion* enter the winter diapause?, 99.

BUTLER, L.—A study of size inheritance in the house mouse. II. Analysis of five preliminary crosses, 154.

CAMERON, M. L.—See McCarter, J .A.

CHOQUETTE, L. P. E.—*Dirofilaria desportesi* sp. nov., a filariid from the black bear in Canada, 344.

CHURCH, N. S. and SALT, R. W.—Some effects of temperature on development and diapause in eggs of *Melanoplus bivittatus* (Say) (Orthoptera: Acrididae), 173.

COMMON, R. H.—See Phillips, W. E. J.

COTTLE, M. K.—See Hoar, W. S.

DAVIES, D. M.—The population and activity of adult female black flies in the vicinity of a stream in Algonquin Park, Ontario, 287.

DUBREUIL, R.—See Auclair, J. L.

ELIGH, G. S.—Factors influencing the performance of the precipitin test in the determination of blood meals of insects, 213. See West, A. S.

FISHER, R. W.—The importance of the locus of application on the effectiveness of DDT for the house fly, *Musca domestica* L. (Diptera: Muscidae), 254.

GRENIER, F.—See Kleerekoper, H.

HART, J. S.—Use of daily metabolic periodicities as a measure of the energy expended by voluntary activity of mice, 83. Effects of temperature and work on metabolism, body temperature, and insulation: Results with mice, 90.

HAYES, F. R.—See McCarter, J. A.

HENSON, W. R. and SHEPHERD, R. F.—The effects of radiation on the habitat temperatures of the lodgepole needle miner, *Recurvaria milleri* Busck (Gelechiidae: Lepidoptera), 144.

HOAR, W. S. and COTTLE, M. K.—Dietary fat and temperature tolerance of goldfish, 41. Some effects of temperature acclimatization on the chemical constitution of goldfish tissues, 49.

HOAR, W. S., MACKINNON, D., and REDLICH, A.—Effects of some hormones on the behavior of salmon fry, 273.

JACOBSON, L. A.—Effects of starvation on larvae of the pale western cutworm, *Agrotis orthogonia* Morr. (Lepidoptera: Phalaenidae), 194.

JODREY, L. H.—See McCarter, J. A.

KLEEREKOPER, H. A new apparatus for the study of sedimentation in lakes, 185.

KLEEREKOPER, H. and GRENIER, F.—The bottom sediments of Lake Lauzon, Montcalm County, Province of Quebec. I. Chemical and physical investigations, 219.

MCCARTER, J. A., HAYES, F. R., JODREY, L. H., and CAMERON, M. L.—Movement of materials in the hypolimnion of a lake as studied by the addition of radioactive phosphorus, 128.

MCCRAW, B. M.—Observations on the development of *Lymnaea palustris* Müller, 378.

MACKINNON, D.—See Hoar, W. S.

MCLEOD, J. A. and BONDAR, G. F.—Studies on the biology of the muskrat in Manitoba. Part I. Oestrous cycle and breeding season, 243.

MALTAIS, J. B. and AUCLAIR, J. L.—Occurrence of amino acids in the honeydew of the crescent-marked lily aphid, *Myzus circumflexus* (Buck.), 191.

MAW, W. A.—See Phillips, W. E. J.

MOORE, J. E.—The entomostraca of Southern Saskatchewan, 410.

NURSALL, J. R.—The early development of a bottom fauna in a new power reservoir in the Rocky Mountains of Alberta, 387.

PELLUET, D.—See Bridgeford, H. B.

PHILLIPS, W. E. J., COMMON, R. H., and MAW, W. A.—Some effects of progesterone on the response of the immature pullet to estrogen, and some observations on the effects of gonadal hormones on the avian spleen and kidney, 201.

PIELOU, D. P. The nonmutagenic action of *p–p'*–DDT and 2-hexachlorocyclohexane in *Drosophila melanogaster* Meig. (Diptera: Drosophilidae), 375.

REDLICH, A.—See Hoar, W. S.

SALT, R. W.—Some aspects of moisture absorption and loss in eggs of *Melanoplus bivittatus* (Say), 55. See Church, N. S.

SHEPHERD, R. F.—See Henson, W. R.

STEPHENS, J. M.—Disease in codling moth larvae produced by several strains of *Bacillus cereus*, 30.

TAYLOR, S. J.—Vascularity of the hypophysis of lower vertebrates. The painted turtle, *Chrysemys picta marginata* Agassiz, 134.

WELLINGTON, W. G.—Air-mass climatology of Ontario north of Lake Huron and Lake Superior before outbreaks of the spruce budworm, *Choristoneura fumiferana* (Clem.), and the forest tent caterpillar, *Malacosoma disstria* Hbn. (Lepidoptera: Tortricidae; Lasiocampidae), 114.

WEST, A. S. and ELIGH, G. S.—The rate of digestion of blood in mosquitoes. Precipitin test studies, 267.

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Contents

Page

Induced Changes in the Cells of the Ovotestis of the Slug, *Deroceras reticulatum* (Müller), With Special Reference to the Nucleolus—*Hope B. Bridgeford and D. Pelluet* - - - - - - - - - - 323

A Note on the Chiggers (Trombiculidae) of Alberta—*John H. Brown and James M. Brennan* - - - - - - - - - - - - 338

Dirofilaria desportesi sp. nov., a Filariid from the Black Bear in Canada —*Laurent P. E. Choquette* - - - - - - - - - - - 344

The Haemocytes of the Mediterranean Flour Moth, *Ephestia kühniella* Zell. (Lepidoptera: Pyralididae)—*J. W. Arnold* - - - - - - 352

Effects of Certain Fumigants on Haemocytes of the Mediterranean Flour Moth, *Ephestia kühniella* Zell. (Lepidoptera: Pyralididae)—*J. W. Arnold* - 365

The Nonmutagenic Action of *p*-*p*′-DDT and γ-Hexachlorocyclohexane in *Drosophila melanogaster* Meig. (Diptera: Drosophilidae)—*D. P. Pielou* - 375

Observations on the Development of *Lymnaea palustris* Müller—*Bruce M. McCraw* - - - - - - - - - - - - - 378

The Early Development of a Bottom Fauna in a New Power Reservoir in the Rocky Mountains of Alberta—*J. R. Nursall* - - - - - - 387

The Entomostraca of Southern Saskatchewan—*J. E. Moore* - - - - 410

Contents of Volume 30 - - - - - - - - - - - 451